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Establishing a baseline value for urinary arsenic:selenium ratio in unexposed populations in the United Kingdom

Eid I. Brima [a], Chris F. Harrington [b], Richard O. Jenkins [c], Andrew G. Gault [d], David A. Polya [e], Gareth F. Pearson [f], Gillian M. Greenway [g] and Parvez I. Haris [c,*]

[a] *Department of Chemistry, Faculty of Science, King Khalid University, Abha, Saudi Arabia*
[b] *Trace Element Laboratory, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK*
[c] *School of Allied Health Sciences, Faculty of Health and Life Sciences, De Monfort University, Leicester, UK*
[d] *Department of Geological Sciences and Geological Engineering, Millar Hall, Queen's University, Kingston, ON, Canada*
[e] *School of Earth, Atmospheric and Environmental Sciences & Williamson Research Centre for Molecular Environmental Sciences, The University of Manchester, Manchester, UK*
[f] *MEP Instruments, Boronia Park, NSW, Australia*
[g] *Department of Chemistry, The University of Hull, Hull, UK*

Abstract. The relationship between arsenic (As) and selenium (Se) in the human body is poorly understood. We have investigated the concentrations of urinary As and Se in three ethnic groups ($n = 63$) in the United Kingdom and show that there is a positive correlation ($r = 0.62$, $p < 0.001$) between total concentrations of As and Se and that the ratio of these two elements is stable, with a mean value ($\pm$SD) of 0.7 ± 0.4. Furthermore, concentrations of individual arsenic species methylarsonate (MA), dimethylarsinate (DMA) and arsenobetaine (AB) in the urine samples show a positive correlation with total Se (As(III) and As(V) were not detected). The intra-individual variation of the As:Se ratio also remains stable over time, as determined by monitoring a volunteer over a period of one year, and deviates only after recent seafood consumption. It appears that the ratio is also stable across diverse populations across different cultures and continents, evident from our calculation of As:Se ratio from concentrations of these elements found in urine samples from different populations published in the literature. Our study involved analysis of 63 urine samples from three ethnic groups (White Caucasian $n = 20$, Asian $n = 21$ and Somali $n = 22$), 58 urine samples from 29 Ramadan fasting volunteers and 12 from one volunteer whose urine samples were collected over a period of one year. All the participants completed a lifestyle questionnaire and were asked to refrain from eating seafood or fish for three days prior to collection of the sample. Total As and Se in urine were determined by inductively coupled plasma mass spectrometry (ICP-MS). As species (AB, DMA, MA, As(III), As(V)) were determined by using high performance liquid chromatography (HPLC) combined with ICP-MS. Mean $\pm$ SD As:Se ratios of 0.8 ± 0.4, 0.7 ± 0.4, 0.4 ± 0.2, 0.7 ± 0.3 and 1.2 ± 0.3 were obtained for the Asian, White Caucasian, Somali, fasting, and one volunteer respectively, giving an overall mean of 0.7 ± 0.4 (SD). It is noteworthy, that when comparing ethnic differences, the Somali group shows a statistically significant lower As:Se ratio (0.4 ± 0.2, $p < 0.05$) compared to Asian and White Caucasian groups; this is ascribed to lower urinary As concentrations in this group. The study over one year with a single volunteer revealed that recent (within 3 days) seafood consumption results in a significantly different ($p < 0.05$) As:Se ratio (4.0). We have calculated from the literature the value of As:Se for populations, exposed to As through drinking water, can range from 2.0–9.6. Based on our own work and the values we calculated from other studies we suggest that the baseline range for mean As:Se ratio is 0.4–1.2, provided that the urine samples

*Corresponding author: Dr. P.I. Haris, School of Allied Health Sciences, Faculty of Health and Life Sciences, De Monfort University, Leicester, LE1 9BH, UK. E-mail: pharis@dmu.ac.uk.

are collected in the absence of recent consumption of seafood. The relatively stable As:Se ratio observed in this study suggests a link between these two elements in humans adding support to earlier studies with animals and humans exposed to inorganic arsenic in drinking water which reported interaction between these elements and that Se may play a role in counteracting As toxicity.

Keywords: Ratio, Ramadan, fasting, ethnicity, urine, arsenic, selenium, baseline, toxicity

1. Introduction

The metabolism and toxicity of the metalloids, arsenic (As) and selenium (Se), have been reported to be modulated by each other [17,38]. Glutathione (GSH) and S-adenosylmethionine are necessary for their reductive metabolism and methylation, respectively [38]. The metabolism and toxicity of inorganic and organic Se compounds has been shown to be modified by inorganic As [17]. The effects of selenite (Se IV) have been demonstrated to modify the toxicity and metabolism of arsenite (As(III)) [38]. These authors suggested that the concomitant exposure to Se IV may enhance the toxic effects of inorganic arsenic. Although As and Se have some similarities, they have very different impacts on the human body. Thus, Se is considered as an essential micronutrient [29], whereas As is classified as a carcinogen and toxic to humans [2,21].

Exposure to As from occupational or As-contaminated drinking water sources has been linked to different cancers such as skin, lung, liver and bladder [49]. Vascular disease such as black foot disease has also been reported to be caused by long-term exposure to inorganic As in drinking water [14,44]. Inorganic As (arsenite (As(III)) and arsenate (As(V))) is methylated in the human body to methylarsonate (MA) and dimethylarsinate (DMA), through consecutive steps of reduction and oxidative methylation of As [40]. In As-exposed populations, As excreted in human urine generally comprises 10–30% inorganic As, 10–20% MA and 60–80% DMA [16,18]. In addition, other methylated As species in trivalent states, such as methylarsonite (MA(III)) and dimethylarsinite (DMA(III)), have been reported among exposed populations [46]. These trivalent methylated As species are considered to be more toxic than inorganic As [20].

Selenium is considered an essential trace element in the human diet, being a component of glutathione peroxidase, which is known to be an effective antioxidant in the human body and acts against the effect of free radical species [34,45]. Selenium has a protective role against cancer as demonstrated by different studies [4,12]. Dietary organic and inorganic Se forms can be transformed in the human body to selenide, which can then be excreted and/or further utilized in selenoprotein synthesis [41]. Inorganic forms of Se are selenite and selenate; organic forms are seleno-amino acids such as selenocysteinyl (SeCys) and selenomethionine (SeMet) [42]. Most of the Se consumed by humans is excreted in urine, thus the amount of Se in urine reflects dietary intake. After stepwise methylation, Se is excreted in urine as monomethylated selenium and trimethylelenium, while dimethylselenide is exhaled in the breath [26]. Various foods are considered as dietary sources of Se, such as Brazil nuts, grain, wheat, cucumber, mushroom, crab, liver and shellfish [30,35]. Deficiency of Se intake results in different diseases such as Keshan's disease, numbness in the legs and arms, brittle hair and deformed nails [3], while excessive Se shows toxic symptoms such as neuromuscular symptoms and skin lesions [1].

Se has been reported to have a positive role against As toxicity, because the two elements act as metabolic antipodes [36]. Urinary Se was reported to associate with an increased proportion of DMA and a decrease in inorganic As in human urine [11]. It has been reported that Se and As counteract the toxicity of each other [24]. In another study with humans, Se was shown to be an effective treatment

against arsenism, a disease that results from long-term exposure to high As in the environment [48]. After administration of 100–200 μg Se/day to patients and control groups for 14 months, the patients showed decreased concentrations of As in blood, hair and urine compared to those of the control group [48]. This would suggest that for health risk assessment among populations exposed to As, Se measurement is also important to estimate the extent of As toxicity.

Whilst some studies have been reported on the relationship between As and Se, they have been limited to studies of arsenic exposed populations [11,19]. The study with exposed populations demonstrated a positive correlation between As and Se in urine and hair [19,37] although one study [27] suggested that there was negative correlation between both elements. In this study, our aim was to see if there is a correlation between urinary As and Se in populations in the UK that are not exposed to high concentration of As, and evaluate the stability of the As:Se ratio. The study included members from different ethnic groups, fasting volunteers and a volunteer whose urinary As and Se concentrations were monitored over a period of one year. The objective of the study was not only to better understand the relationship between As and Se in unexposed populations, but also to explore the possibility of developing an additional biomarker for evaluating As exposure in humans.

2. Experimental section

2.1. Chemicals and reagents

Deionised water ($>18\,\Omega\,\mathrm{cm}^{-1}$) was used throughout the study. Stock solutions of arsenic species were prepared and calibrated against an As(V) standard (1000 ± 3 mg/l, CPI, International, USA). A Se stock solution standard was purchased from Sigma-Aldrich (Germany); As(III) ((As_2O_3), Sigma-Aldrich, Germany) was dissolved in 4 g/l sodium hydroxide and made up to appropriate volume with 2% v/v HNO_3 (UPA, Romil, UK); DMA ($(CH_3)_2AsOOH$, Sigma-Aldrich, Germany); MA ($(CH_3)_3AsO(OH)_2$, Greyhound, Dorset, England) and AB ($(C_5H_{11}AsO_2)$, Fluka, Fisher Chemicals, UK). In addition, 10 μg/l of yttrium (PlasmaCAL, Québec, Canada) was used as an internal standard for total As and Se analysis by ICP-MS.

The IC-ICP-MS mobile phase (20 mM NH_4HCO_3) was prepared by dissolving an appropriate amount of ammonium hydrogen carbonate (Fisher Chemicals, UK) in 950 ml of deionised water, adjusted to pH 10.3 with 35% ammonia (Fisher Chemicals, UK), filtered through a 0.45 μm membrane filter before adding 50 ml of methanol HPLC grade (Fisher Scientific, UK) and finally degassed with helium. The mobile phase, with slight modification, was used previously by Pedersen et al. [32]. Germanium solution was added to the mobile phase as an internal standard to a final concentration of 50 μg/l.

2.2. Instrumentation

Determination of total As and Se in urine was performed using an ELAN DRCII ICP-MS (PerkinElmer SCIEX, Concord, Ontario, Canada). Signals at m/z 75 and 82 were monitored, and the instrumental conditions were as follows: radio frequency power 1350 W; gas flows, plasma 15 l/min, auxiliary flow 1.20 l/min, nebulizer flow 0.97 l/min; with nickel sampler and skimmer cones. $^{35}Ar^{40}Cl$ signal was reduced by applying the correction Eq. (1), and Se interference was also removed as shown in Eq. (2):

$$^{75}\mathrm{As} = {}^{75}\mathrm{As} - 3.1288191 * ({}^{77}\mathrm{Se} - 0.8739977 * {}^{82}\mathrm{Se}), \tag{1}$$

$$^{82}\mathrm{Se} = {}^{82}\mathrm{Se} - 1.007833 * {}^{83}\mathrm{Kr}. \tag{2}$$

Urinary As speciation was determined by HPLC-ICP-MS. The HPLC system comprised a 790 Personal IC chromatograph (Metrohm, Switzerland) liquid chromatography solvent delivery pump, fitted with a 100 µl sample loop, and an anion exchange column Hamilton PRP-X 100 (250 × 4.1 mm i.d.) with a guard column (4 × 3 mm i.d., Phenomenex, Polymerx RP-1). The flow rate of the mobile phase was 1 ml/min. The ICP-MS was used as a chromatographic detector. The outlet of the HPLC system was coupled directly with PEEK i.d. 90 µm tubing to the inlet of the ICP nebulizer. The signals at m/z 75, 77 and 51 were monitored in the graphic mode. The m/z 51 and 77 were used to monitor the $^{35}Cl^{16}O^{+}$ and $^{40}Ar^{37}Cl^{+}$ interferences, respectively. The chromatographic and instrumental conditions for As speciation were as follows: ICP-MS (PQ II, VG Instruments, Winsford, UK); radio frequency power 1350 W; gas flows, plasma 13 l/min, auxiliary flow 0.95 l/min, nebulizer flow 0.94 l/min; and nickel sampler and skimmer cones.

2.3. Sample collection and preparation

Urine sample collection and storage were carried out as reported in our previous studies [5,6,8]. All volunteers (ethnic groups and fasting group) were asked to refrain from eating fish and seafood for three days prior to sample collection, and to complete a questionnaire which gathered information on age, gender and ethnicity along with lifestyle. The questionnaire was accompanied with a letter explaining the objective of the study and how to deal with the urine samples in terms of collection and storage. All procedures followed were in accordance with the ethical guidelines of the Research Ethics Committee, Faculty of Health and Life Sciences, De Montfort University. Urine samples were collected directly into polyethylene bottles (Fisher, UK). The normality of each sample was checked by using Combur^{9}-test (Roche, Germany) urine test strip. The samples were kept in the freezer at -20°C until the analyses were carried out. Prior to analysis, the samples were filtered through 0.45 µm syringe filter and diluted up to 5-fold with 2% HNO_3 for total As and Se determination.

In total 133 urine samples were collected from different groups in this study. The total number of volunteers were 93 adults (mean ± SD age 31.8± 10.3 years (five volunteers did not report their age); 69 men and 24 women). Midstream first morning void urine samples were collected from three ethnic groups ($n = 63$): Asian ($n = 21$), Somali ($n = 22$) and White Caucasian ($n = 20$). The Ramadan fasting (RF1 and RF2) group were paired samples, and collected from the same individuals at the beginning of fasting period (RF1) and at the end of fasting period (RF2). For the Ramadan fasting urine samples ($n = 58$), a midstream first morning void urine samples was collected at the beginning of the fast (RF1, $n = 29$) and a midstream first sunset void urine sample at the termination of an approximately 12 h long fast was also collected from the same volunteers (RF2, $n = 29$) (see [6] for further details). Twelve urine samples were collected from one volunteer over the course of a year without (volunteer part A, $n = 6$) and with (volunteer part B, $n = 6$) seafood consumption. All the subjects in the study were resident in the city of Leicester, UK during the course of the investigation and the As in their drinking water was below the level of detection.

2.4. Quality control

A certified reference material (CRM) from the National Institute of Environmental Studies (NIES), Japan, was used to validate the total As and total Se methods. The material was reconstituted as described by the manufacturer. A spiking experiment for both total As and Se, and As species in urine was carried out and the results indicated that any interference present in the urine samples had no effect on the accuracy of the total As or Se measurements, or the As speciation analysis.

2.5. Determination of creatinine

Creatinine was analysed photometrically by using a Metra Creatinine Assay Kit (Quidel Corporation, USA). The actual concentrations of As and Se in urine for all groups (ethnic groups, fasting group and volunteer's samples) were expressed as μg element/g creatinine.

2.6. Statistical analysis

One-way ANOVA was used to identify significant differences for comparisons involving all three groups, with post-hoc analysis by the Tukey HSD test to identify significance differences for pair-wise comparisons. The association of demographic variables such as tea, coffee, soft drink, alcohol, smoking, age and gender were also evaluated. These factors were evaluated regarding total As and total Se in urine. The difference between RF1 and RF2 in terms of total As, Se concentrations and As:Se ratio was tested by using the Paired Student's t-test, because these samples were collected from the same person twice.

3. Results

In this study, unexposed groups refer to populations who are not exposed to a high concentration of As through drinking water. Thus for example, the UK population investigated in this study are not exposed to high concentration of As through drinking water and are referred to as "unexposed".

The pH of all urine samples studied was within the range of 5–8. This is within the normal range (4.5–8.0) expected for the human urine range referred to by Chen et al. [10]. The method for total and speciation analysis of As in urine samples were reported in our previous work [5,6]. Therefore, the results of total As and As species, especially for ethnic group were reported again accompanied with Se results, because this study focused on the relation between As and Se in human urine.

3.1. Total As and Se analysis in urine samples

The limit of detection (LOD) and the limit of quantification (LOQ) for total As and Se were calculated by measuring the blank ten times, the LOD ($3 \times$ SD) was 0.05 μg/l for As and 0.19 μg/l for Se, and the LOQ ($10 \times$ SD) was 0.18 μg/l for As and 0.63 μg/l for Se. The total As concentration and Se in the human urine CRM was found to be 140.7 ± 1.9 μg/l and 67.9 ± 4.5 μg/l compared to the certified value of 137 ± 11.0 μg/l and 59.0 ± 5.0 μg/l, respectively. Simultaneous measurement of total As and Se were validated by spiking experiment and the recovery was 103% ($n = 9$) for As and 102% ($n = 9$) for total Se. The spiking experiment was carried out by using 50 μg/l of each element in urine sample, then diluted (5-fold) with 2% v/v HNO_3 to achieve 10 μg/l total spiked concentration. The calibration curves for total As and total Se were drawn within the range (1–20 μg/l) and gave r^2 values of 0.9999 or better during the course of the study. The accuracy and reproducibility of the method was validated by measuring 10 μg/l standard of both As and Se after each 20 runs. The reproducibility [between-run, $n = 10$; replicates ($n = 3$) for each measurement] was 10.3 ± 0.3 μg/l (2.6% RSD) for Se and 10.5 ± 0.2 μg/l (1.4% RSD) for As.

3.2. Arsenic speciation analysis in urine samples

The actual concentrations of As species detected in all different groups (ethnic and fasting groups) were expressed in μg As/g creatinine, before they were presented in percentages. The calculation was

carried out from raw instrumental data using the in-house Turbo Pascal programme DBSCORR Version 8 [33].

For speciation analysis a spiking experiment was carried out and recoveries of the arsenic species that were added to the urine sample was AB (90%), DMA (95%), As(III) (86%), MA (95%), and As(V) (98%). The CRM No. 18 was used to validate the method and the results were as follows: AB 69.7 ± 1.4 μg As/l and DMA 38.4 ± 0.6 μg As/l; the certified values were 69 ± 12 μg/l and 36 ± 9 μg/l, respectively. The problem of interference from chloride present in urine was overcome and has been discussed in our previous publication [5,6], when the separation of the five arsenic species (AB, DMA, As(III), MA and As(V)) was carried out by using HPLC-ICP-MS.

3.3. Total As, Se, As species, correlation and As:Se ratio in urine samples

The concentrations of As, Se and As:Se ratios for the ethnic groups (Asian, Somali and White Caucasian), the fasting group (paired samples), and the one-year study volunteer are shown in Tables 1, 2 and 3, respectively. Table 1 shows combined total (mean ± SD) urinary As and Se concentrations for the three ethnic groups (total As = 17.4 ± 13.5 μg/g creatinine and total Se = 28.0 ± 15.1 μg/g creatinine). Table 2 shows total (mean ± SD) urinary As and Se concentrations for the three fasting group (total As = 18.6 ± 13.4 μg/g creatinine, total Se = 28.8 ± 15.4 μg/g creatinine). Table 3 shows the results of one volunteer who was monitored for one year for total urinary As and Se concentrations, and shows the effect of seafood consumption on these concentrations (total As = 25.4 ± 19.5 μg/g creatinine and total Se = 8.7 ± 0.7 μg/g creatinine).

Comparison across the three groups by ANOVA revealed that there was a statistically significant difference between the groups for total As ($p < 0.05$), total Se ($p < 0.05$) and the As:Se ratio ($p < 0.05$). With post-hoc testing for significance, the Somali group showed a significant difference ($p < 0.05$) when compared with either the Asian or White Caucasian groups, regarding total As or the As:Se ratio

Table 1

Total arsenic, total selenium and As: Se ratio, in human urine among three different ethnic groups: Asian, Somali and White Caucasian

	Group (n)	Mean	SD	Median	Min.	Max.
As (μg/g creatinine)	Asian (21)	20.6	17.5	15.4	5.7	84.4
	Somali (22)	7.2	3.8	6.5	0.4	14.8
	White Caucasian (20)	24.5	19.3	17.6	6.4	71.2
Se(μg/g creatinine)	Asian (21)	25.1	9.0	23.5	12.4	46.1
	Somali (22)	22.2	23.2	19.6	0.7	121.6
	White Caucasian (20)	36.6	13.2	33.9	15.5	63.7
As:Se	Asian (21)	0.8	0.4	0.6	0.2	1.8
	Somali (22)	0.4	0.2	0.4	0.1	0.7
	White Caucasian (20)	0.7	0.4	0.5	0.3	1.5
P-values		Total As	Total Se	As:Se ratio		
	Asian (21) vs. Somali (22)	<0.05	ns	<0.01		
	Asian (21) vs. White Caucasian (20)	ns	ns	ns		
	Somali (22) vs. White Caucasian (20)	<0.01	<0.05	0.09 (<0.05*)		

P-values are also presented (ns = not significant).
* Student t-test was used.

Table 2

Total arsenic, total selenium and, As:Se ratio, in human urine among fasting group (RF): two types of urine samples RF1* and RF2*

	Group (n)	Mean	SD	Median	Min.	Max.
As (μg/g creatinine)	RF1 (29)	18.3	11.8	15.7	5.7	54.9
	RF2 (29)	17.7	12.5	14.6	2.1	66.8
Se (μg/g creatinine)	RF1 (29)	26.2	11.7	23.0	7.8	61.8
	RF2 (29)	29.6	15.2	25.8	3.1	83.4
As:Se	RF1 (29)	0.7	0.3	0.7	0.2	1.4
	RF2 (29)	0.7	0.6	0.5	0.2	3.4
P-values		Total As	Total Se	As:Se ratio		
	RF1 (29) vs. RF2 (29)	ns	ns	ns		

P-values are also presented (ns = non significant).
*RF1 and RF2 urine paired samples were collected from the same person at the beginning and at the end of fasting period, respectively.

Table 3

Total As and total Se (μg/g creatinine) in urine of one volunteer monitored for one year

	Samples (n)	Mean	SD	Median	Min.	Max.
As (μg/g creatinine)	Volunteer (without fish) part A (6)	11.6	3.5	13.2	4.6	13.7
	Volunteer (with fish) part B (6)	39.2	29.0	25.4	18.4	94.6
Se (μg/g creatinine)						
	Volunteer (without fish) part A (6)	9.2	1.7	9.5	6.7	11.2
	Volunteer (with fish) part B (6)	8.2	2.7	8.0	4.7	12.7
As:Se	Volunteer (without fish) part A (6)	1.2	0.3	1.3	0.7	1.6
	Volunteer (with fish) part B (6)	4.9	3.2	3.0	2.7	10.1
P-value		Total As	Total Se	As:Se ratio		
	without fish Part A (6) vs. with fish Part B (6)	<0.05	0.45	<0.05		

Notes: The data are divided into two groups depending on whether seafood was ingested shortly before urine sample collection. Volunteer part A (no recent seafood $>$ 1 week ago), Volunteer part B (recent seafood $\leqslant$ 2 days ago).

(Table 1). No statistically significant ($p > 0.05$) difference was found for any of the three parameters when comparing Asian and White Caucasian groups. Table 2 shows there is no influence in the total As, total Se and As:Se ratio for volunteers whose urine was collected before and after an approximately 12 h long fast. From Table 3, it can be seen that a significant difference ($p < 0.05$) regarding As:Se is seen for the one year volunteer when the values are compared before and after seafood consumption.

The distribution of total As, total Se and As:Se ratio of the different ethnic groups (Asian, Somali and White Caucasian) are shown in Fig. 1. In all three boxplots (Fig. 1), the Somali group shows less variation than the Asians and White Caucasians. These latter two groups show more distribution in all boxplots. Outliers only appeared on upper values for both Asian and White Caucasian in total As concentration. Only the Somali group showed outliers on upper and lower values for total Se concentration. Correlations between total As and total Se in urine samples of different ethnic groups (Asian, Somali and White Caucasian), fasting groups and the single volunteer are shown in Fig. 2. Urinary total As concentration was positively linearly correlated with the urinary total Se concentration. The correlation

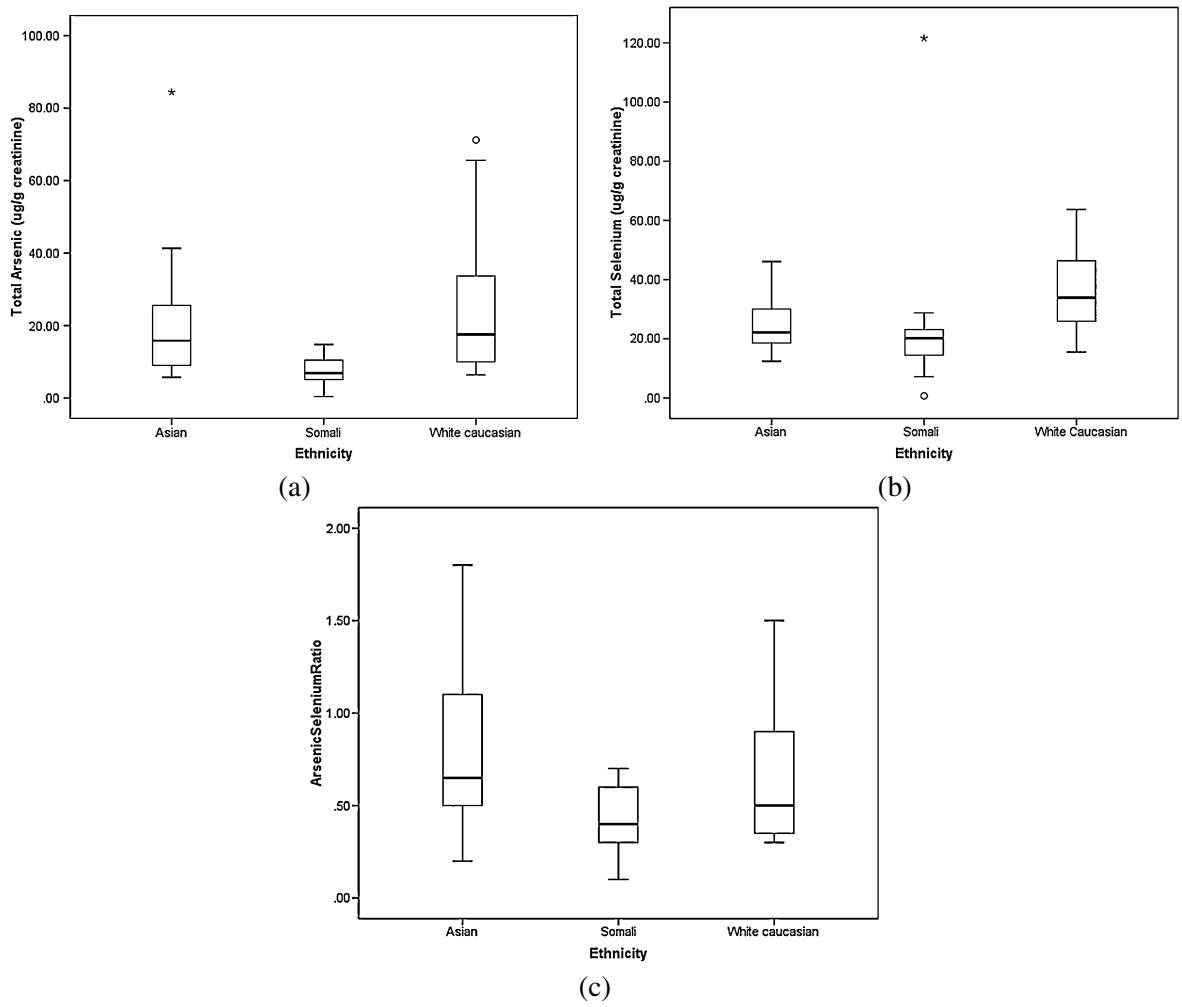

Fig. 1. Boxplots for the three ethnic groups (Asian, Somali and White Caucasian); total arsenic (A), total Se (B) and As to Se ratio (C).

coefficients for Asian, Somali, White Caucasian, fasting group (RF1 and RF2) and the single one year volunteer (volunteer part A and volunteer part B) are in the range of 0.5–0.7 (Fig. 2).

The As:Se ratios in urine samples for different groups are shown in Fig. 3. As:Se ratios were similar for all the different groups: Asian 0.8 ± 0.4, White Caucasian 0.7 ± 0.4 and the Fasting group: 0.7 ± 0.5, except for Somali 0.4 ± 0.2. The As:Se ratios showed significant difference as a function of ethnicity. The Somali group displays the lowest As:Se ratio, indicating that the level of excreted Se is higher than that of As, which is true for all ethnic groups. However, the exceptional situation for the Somali group is that the level of Se is 3.1-fold higher than that of As, in contrast to 1.3 and 1.5-fold in the Asian and White Caucasian groups, respectively. The As:Se ratios for Asian and White Caucasian groups are virtually identical (mean = 0.8). We also calculated As:Se ratios from the literature from different countries: UK [47], Germany [15], Brazil [13], China [50,51], Taiwan [9] and Croatia [39] and Italy (Claudia Cascio, personal communication). The As:Se ratios obtained are in agreement with what we have found for the UK population.

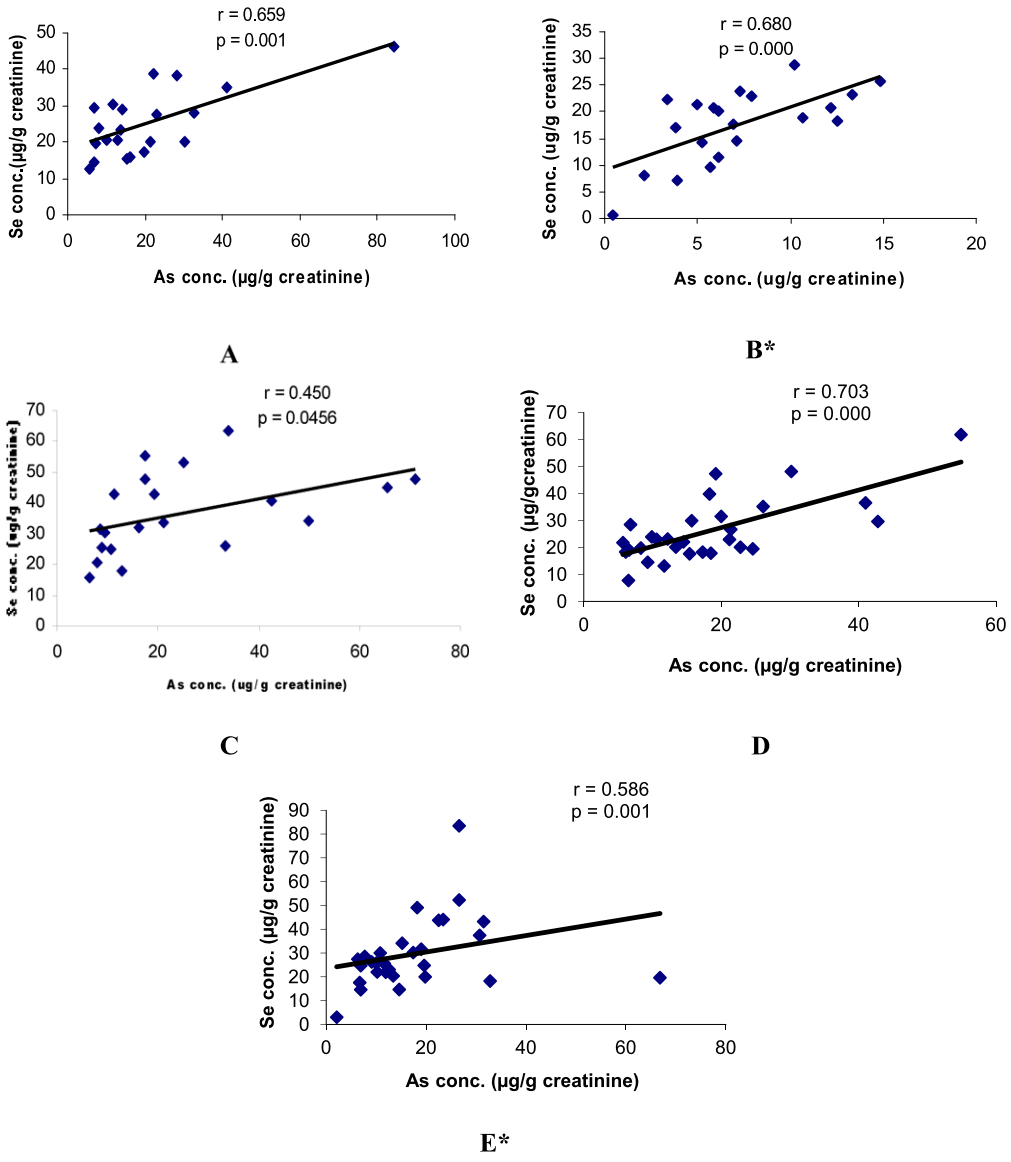

Fig. 2. Correlations of total As and total Se in urine samples of different groups: ethnic groups (Asian (A), Somali (B) and White Caucasian (C)), fasting group: RF1 (D) and RF2 (E). $^{*}r$ values for Somali and RF2 are shown after outlier removal. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/BSI-130046.)

For one volunteer we collected urine samples over one year, without (volunteer part A, no seafood consumption for more than one week) and with seafood consumption (volunteer part B, seafood consumption for less than three days). The ratio of As:Se for the volunteer remains stable (part A 1.2 ± 0.3) when refraining from seafood consumption. However, this ratio is altered after seafood consumption (part B 4.9 ± 3.2). As shown in Fig. 4 the clearance of As originating from seafood is achieved after 3 days after seafood ingestion. It is obvious from this graph that seafood ingestion has an effect on total As and As:Se ratio, but the Se level is unaffected.

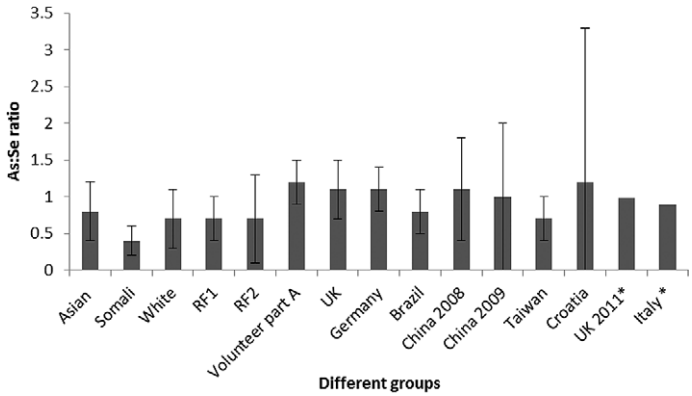

Fig. 3. As:Se ratios (mean ± SD) in urine samples of unexposed populations in UK: Asian, Somali and White Caucasian groups; Ramadan fasting groups: RF1 and RF2; volunteer part A. This is compared with calculated values from the literature studies for unexposed populations from different countries: UK [47], Germany [15], Brazil [13], China [50], China [51], Taiwan [9] and Croatia [39], UK 2011* and Italy* are median values (Claudia Cascio, personal communication).

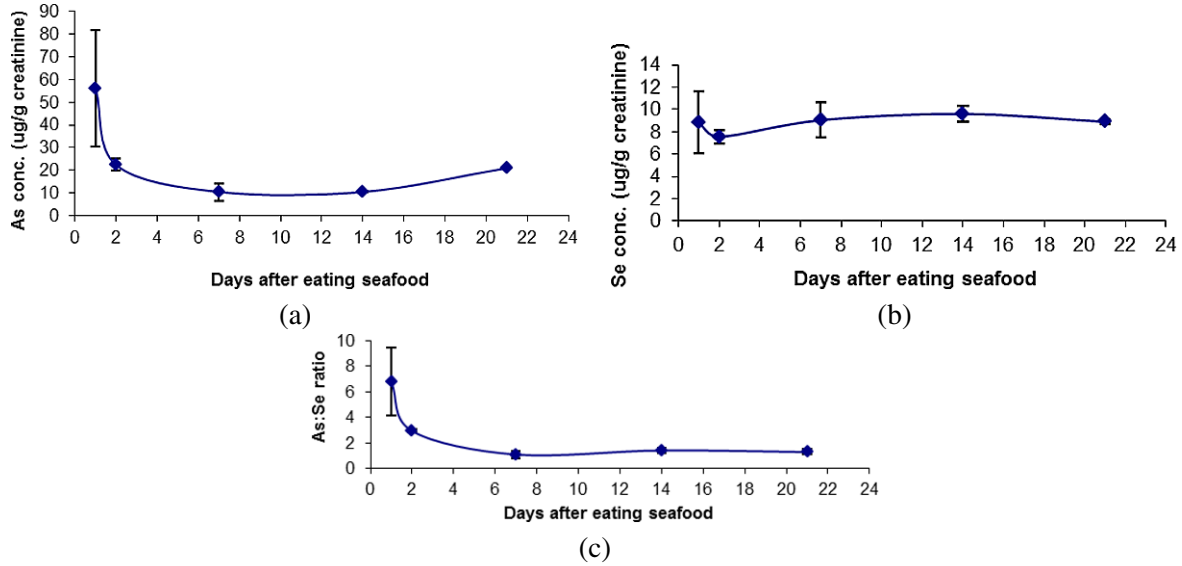

Fig. 4. Total As (a), Se (b) and As:Se ratio (c) in urine samples of one volunteer collected over one year and shows the effect of seafood ingestion on the three values; Mean values ± SD. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/BSI-130046.)

The As:Se ratio in fasting volunteers (0.7 ± 0.5) is similar to that for other volunteers e.g. the Asian (0.8 ± 0.4) and White Caucasian (0.7 ± 0.4) groups, with the exception of the Somali group (0.4 ± 0.2). This suggests that the Ramadan fasting (diurnal fasting) does not result in an overall alteration in As:Se ratio, which is evident from the results for RF1 (0.7 ± 0.3) and RF2 (0.7 ± 0.6).

Table 4 shows the As, Se, and As:Se ratio observed in our study with the ratio we calculated from literature reports of As and Se concentrations in other studies. It is evident from this table that with few exceptions (Somali, Croatia and UK (Indians)), the mean As:Se ratio is between 0.7–1.37 for all studies reported so far regarding As and Se concentrations in human urine for populations that are not exposed to very high concentration of arsenic.

Table 4
As:Se ratios in urine samples of groups exposed and unexposed to As in drinking water

Group	Number of urine samples	As concentration (μg/g creatinine) mean ± SD	Se concentration (μg/g creatinine) mean ± SD	As:Se (wt./wt.) mean ± SD	Reference
Unexposed					
UK (White Caucasian)	20	24.5 ± 19.0	36.6 ± 13.2	0.7 ± 0.4	This work
UK (Asian)	21	20.6 ± 17.5	25.1 ± 9.0	0.8 ± 0.4	This work
UK (Somali)	22	7.2 ± 3.8	22.2 ± 23.2	0.4 ± 0.2	This work
UK (one volunteer, part A)	6	9.3 ± 3.9	9.2 ± 2.0	1.2 ± 0.3	This work
UK (combined ethnic groups)	161	15.2 μg/l[m]	15.5 μg/l[m]	0.98	[7]
UK (White Caucasian)	23	11.2 μg/l[m]	18.9 μg/l[m]	0.59	[7]
UK (Bangladeshi)	54	23.6 μg/l[m]	17.6 μg/l[m]	1.34	[7]
UK (Pakistanis)	21	5.6 μg/l[m]	4.1 μg/l[m]	1.37	[7]
UK (Indians)	23	5.8 μg/l[m]	13.8 μg/l[m]	0.42	[7]
UK (combined Asian)	98	35 ± 7.0 μg/l[m$]	35.5 ± 10.3 μg/l[m$]	0.99 ± 1.5	[7]
UK	200[a]	12.3 μg/l	16.2 (9.2) μg/l	0.8 (1.3) 1.1 ± 0.4	[47]
Italy	37	30 μg/l[m]	33.8 μg/l[m]	0.89	[7]
Germany†	72[b]	10	12	0.8	[15]
Germany†	87[c]	12	11	1.1	[15]
Brazil#	4	28.5 ± 7.0 μg/l	39 ± 7.4 μg/l	0.8 ± 0.3	[13]
China	43	86.0 ± 53.2 μg/l	78.8 ± 43.3 μg/l	1.1	[50]
China	44	0.03 ± 0.01 mg/l	0.03 ± 0.01 mg/l	1.0	[51]
Taiwan	30	1.62 ± 0.17 μg/l	2.46 ± 0.53 μg/l	0.7	[9]
Croatia	25	32.98 (10.17–57.14) 23.49 (μg/kg) (4.65–83.13)	15.55 (10.60–22.78) 19.39 (μg/kg) (4.38–34.58)	2.1 ≠ 1.2	[39]
Exposed					
UK» (one volunteer, part B)	6	37.2 ± 30.6	9.2 ± 3.6	4.0 ± 2.7	This work
Bangladesh	429	181.4 ± 40.2*	20.1 ± 3.8*	9.6 ± 3.8*	[27]
Taiwan	252	96.9 ± 7.3 μg/l	22.4 ± 0.9 μg/l	4.3	[19]
Chile	93	55.8 ± 41.6 μg/l	28.3 ± 13.9 μg/l	2.0	[11]

No recent seafood consumption, personal communication.
» exposed to recent seafood ingestion (⩽2 days ago).
[a] Total As (12.3 μg/l, $n = 23$); total Se (16.2 μg/l, $n = 200$ and 9.2 μg/l, $n = 100$).
† Geometric mean, seafood ingestion was not reported, total As range (1–375 μg/l).
[b] Children.
[c] Adults.
[m] Median values (concentrations have been corrected after adjusting with specific gravity).
[m$] Combined median values for Asian groups.
* The mean and SD were calculated after the data were pooled.
≠ After creatinine adjustment.

The arsenic species percentages of the sum of all species in all different groups are as follows: ethnic groups: Asian (83% AB, 16% DMA and 1% MA); Somali (48% AB, 50% DMA and 2% MA); White Caucasian (77% AB, 22% DMA and 1% MA). These values were reported in our previous work [5]. For the fasting group, the species percentages [6] was as follows: RF1 (68% AB, 29% DMA and 3%

MA); RF2 (60% AB, 34% DMA and 6% MA). Arsenic species concentration were positively linearly correlated with the urinary total Se concentration. The correlation coefficients for Asian, Somali, White Caucasian, fasting group were as follows: AB: $r = 0.6$, 0.1, 0.2, 0.2, respectively; DMA: $r = 0.8$, 0.2, 0.5 and 0.4, respectively; since MA was not detected in more than two samples in each ethnic group it was only reported for fasting group $r = 0.4$.

Different demographic variables, daily habits such as tea, coffee, soft drink, alcohol and smoking, in addition to gender and age were investigated to explore their potential correlation with urinary As and Se concentrations. The influence of these different demographic variables was assessed for all the groups studied. There were no significant differences ($p > 0.05$) resulting from any of these demographic variables.

4. Discussion

Previous studies [11,19] have established a positive correlation between total urinary As and total urinary Se in populations exposed to As in their drinking water, with the exception of one study [27] which reported a negative correlation. It was pointed out by Christian et al. [11] that this negative correlation could be due to the fact that the latter study lacked adequate control of a range of factors with the exception of gender. Speciation analysis was carried out by [19] and Christian et al. [11] and they also found a positive correlation between inorganic As and % DMA and Se. However, correlation between urinary As and Se in populations that are not exposed to high concentrations of inorganic arsenic has not been investigated. Our study was aimed at obtaining information in this area and also to explore if there is a baseline ratio for the concentrations of these two elements in urine.

We show for the first time that, in line with what has been reported for populations that are exposed to high levels of arsenic [11,19], there is a positive correlation between total As and Se and also between Se and the various As species (with the exception of inorganic As, which was not detected in our study) for populations that are not exposed to high concentrations of inorganic As. This finding suggests that there is no difference between exposed and unexposed populations regarding the correlation between Se and As in human urine as they both show similar trends. Besides the positive correlation with total As and Se, we also found a positive linear correlation between Se and AB and DMA among the different groups in this study. Furthermore, our study for the first time has shown a correlation between Se and AB in human urine among unexposed populations.

Besides agreement with the studies by Hsueh et al. [19] and Christian et al. [11] with exposed populations, our findings are also consistent with the detection of a positive correlation between Se and Hg in marine animals [43]. A positive correlation between Se and Hg has been reported in the liver and kidney of the harbour porpoise. A positive correlation exists between Se and toxic elements in both humans and animals.

Another interesting finding of our study is that the As:Se ratio is virtually identical not only for the different groups studied here but also across these diverse populations spanning different cultures and continents (e.g. UK study with 200 volunteers, German study with 159 volunteers, Chinese studies with 87 volunteers (44 and 43 volunteers), study from Taiwan with 30 volunteers, study from Brazil with 4 volunteers). A virtually identical ratio was obtained for a volunteer for a period of one year. Indeed, the As:Se ratio is also stable within a single day as was evident from the analysis of urine samples collected at the beginning of a fast (RF1) and after an approximately 12 h fast (RF2). Although the specific concentration of As and Se varies significantly between the different groups for unexposed

population, the ratio of As:Se determined is found to be virtually identical with the exception of the Somali group. The low As:Se ratio for the Somali group (0.4) can be explained as being due to the relatively low level of As in their urine, but they show higher concentrations of As in their fingernails [5]. The ratio of As:Se (0.7) in Asian and White Caucasian populations observed by us and calculated from a previous study of 200 people [47] suggests that the relative ratio of these two elements is stable in the general UK population and may be a more important parameter than the actual concentrations of the two elements. The calculated ratios of As:Se from different countries for exposed and unexposed population is detailed in Table 4. In the case of exposed and unexposed population the concentration of Se does not vary tremendously. However, large variations in As concentrations can be observed because of exposure to As through consumption of fish, high quantities of rice [8] or As contaminated drinking water, which results in variation in the As:Se ratio.

It is tempting to suggest that the As:Se ratio determined in the current study can be considered the baseline level for the As:Se ratio in unexposed populations and can be used as a reliable biomarker of As and Se level in humans rather than relying solely on As or Se concentrations separately. However, the baseline value should be used with caution, because one of the groups (Somali) is significantly different from the groups studied here. Nevertheless, it appears the calculation of the ratio may provide a more complete picture of As and Se status in human urine when used in conjunction with the actual concentration of the two elements coupled with a background knowledge of the individuals recent food intake.

Dietary intake of As and Se may play a role in the observed ratio. It may be possible that similar intake of these two elements, from food and fluids, results in a similar pattern of urinary excretion resulting in us obtaining a ratio of approximately 1.0 for urinary As:Se. The observation that the ratio is also close to one for people from Brazil, China, Taiwan, Italy, Germany suggests that despite geographical and dietary differences the ratio is highly stable although the actual concentrations of As and Se, for example for Brazil, is very different from our study. Although diet can play a role, it is not easy to suggest a biochemical reason for the relatively constant As:Se ratio observed in our study. However, it has been previously reported that the ratio between mercury (Hg) and Se in the liver of various marine mammals (seal, dolphins, and porpoise) is 1.0 [23]. Koeman et al. suggested that this may reflect a protective role of Se in this animal. In further experiments they found that Se at lower concentrations has an antagonistic role, while at higher concentration it has a synergetic role together with Hg. It has been reported that Se and As counteract the toxicity of each other by a mechanism involving reaction of the two elements to form a conjugate in the liver, which is then excreted in the bile [24]. Kenyon et al. [22] carried out a study with mice to investigate whether differing dietary Se status could alter urinary As after exposure to As(V). They concluded that an excessive Se rich diet was associated with excretion of high proportion of urinary inorganic As and low proportion of organic As, compared with a Se sufficient diet. In contrast, a Se deficient diet was found to eliminate As(V), As(III) and DMA in urine more slowly than a Se sufficient diet. These findings lend further support for our study and others which suggest possible interaction between Se and As in humans. If a biochemical process exists in maintaining a ratio of approximately 1.0 between As and Se in urine, then it suggests that As intake may also be important for human health. This would be in agreement with a previous suggestion which indicated that intake of trace amounts of As may be necessary for metabolism [2].

5. Conclusion

This study for the first time demonstrated a positive correlation between urinary As and Se concentrations amongst a UK population which is not exposed to high concentration of inorganic arsenic.

Furthermore, a baseline value for urinary As:Se ratio was established for a UK population and diverse populations across different cultures and continents. The intra-volunteer variation of As:Se ratio remains stable over time, as determined by monitoring a volunteer over a period of one year, and is only affected by recent seafood consumption. Similar ratios are found when we calculated the As and Se ratio from concentrations of these elements reported in a previous study of 200 people in the UK by another group. This agrees with values obtained for populations from China, Germany, Italy, Taiwan and a small group of volunteers from Brazil. Besides seafood consumption, we found that ethnicity can also result in a significant deviation in the As:Se ratio. Seafood consumption and exposure to As through drinking water results in an increase in the ratio to between 2.0 to 9.6. The Somali ethnic group showed a low As:Se ratio (0.4) which can be explained as being due to the lower level of As in their urine. The fact there is a positive correlation between As and Se and also that the ratio of these elements remain virtually constant between different groups, across different continents, strongly supports the previous studies which have reported that these two elements interact in the human body such as the involvement of Se in the methylation of As.

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