Original Article

Urinary arsenic excretion profiles and associated dietary factors in Japanese women from a coastal area in Chiba Prefecture

Cimi Ilmiawati[1,2], Masanori Ogawa[1], Satoshi Sasaki[3], Tomoko Watanabe[4], Fujio Kayama[1]

[1]Department of Environmental and Preventive Medicine, Jichi Medical University, Tochigi, Japan 329-0498
[2]Department of Pharmacology, Faculty of Medicine, Andalas University, West Sumatra, Indonesia
[3]Department of Social and Preventive Epidemiology, School of Public Health, The University of Tokyo
[4]Department of Nutrition, Chiba Prefectural University of Health Sciences

Abstract

Background：Seafood and seaweed are part of the daily diet in Japan. Despite the essential nutrients they provide, concerns have been raised regarding the various forms of arsenic（As）in these foods. Because populations living in coastal areas have better access to seafood and seaweed, it is important to study their level of exposure to As from these dietary sources.
Objectives：To examine the urinary As excretion profiles of and identify dietary factors possibly associated with urinary As in Japanese women living in Choshi, a coastal area in Chiba prefecture.
Methods：Daily nutrient intake was assessed by a diet history questionnaire（DHQ）and urinary As species were determined by high performance liquid chromatography–inductively coupled plasma-mass spectrophotometry（HPLC-ICP-MS）in 92 participants, followed by statistical analyses of the data obtained.
Results：The geometric mean（GM）of total urinary As was 248.3 μg/g creatinine（μg/g cre）. The main As metabolites in the urine were the organic compounds arsenobetaine（AsBe）（GM 163.4 μg/g cre）and dimethylarsinic acid（DMA；GM 49.6 μg/g cre）. The urinary inorganic As（iAs）concentration was low（GM 1.8 μg/g cre）. Analysis on toxicologically relevant As species showed that the proportion of iAs, monomethylarsonic acid（MMA）, and DMA were 3.4%, 3.1%, and 91.6%（GM）, respectively, after removing AsBe and other As species from total As. Spearman's correlation coefficients showed that seafood and seaweed intake was significantly correlated with %iAs（r=-0.34；r=-0.36, respectively）, %MMA（r=-0.25；r=-0.28, respectively）, and %DMA（r=0.33；r=0.37, respectively）. Intake of B vitamins, vitamin C, and soy isoflavones showed significant negative correlations with %iAs and %MMA, but positive correlations with %DMA.
Conclusions：Japanese women from the coastal area of Choshi are exposed to low-level iAs. Their intake of B vitamins and vitamin C was associated with proportions of urinary As species. We identified for the first time associations between estimated soy isoflavones intake and the proportion of urinary As species.
（Keywords：arsenic, intake, Japanese, seafood, seaweed, urine.）

Introduction

Arsenic（As）is a natural element ubiquitously present in the environment. Exposure to As may occur through an occupational source such as non-ferrous ore smelting,[1] by drinking As-contaminated water in areas with geologically high As,[2] or by consuming dietary sources capable of accumulating As from the soil and water such as rice, seafood, and seaweed.[3]

Arsenic presents as both inorganic and organic forms. Inorganic As（iAs）is commonly found in the environment and has been established as a human carcinogen.[2] Exposure to iAs has also been associated with cardiovascular[4] and metabolic diseases.[5] The toxicity profile of As depends on its chemical form and oxidation state. iAs（which presents

Correspondence to：Fujio Kayama, Department of Environmental and Preventive Medicine, Jichi Medical University, Tochigi, Japan 329-0498, E-mail：kayamaf@jichi.ac.jp
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as As^{III} and As^{V}) is more toxic than organic As, and trivalent arsenicals are more toxic than pentavalent arsenicals.[6] Dietary sources are the main route of As exposure in populations without occupational exposure and low As levels in the drinking water (<10 μg/L). While the effect of iAs has been well established, the consequence of chronic exposure to various forms of organoarsenicals from dietary sources is not fully understood.

Seafood, seaweed, rice-based products, meat, poultry, vegetables, and mushrooms have been identified as the main contributors of dietary As in European countries, the United States (US), and Japan,[3, 7-9,] which has raised concern over the consumption of these foods. For example, the United Kingdom Food Standard Agency (UK FSA) issued a warning to avoid consuming hijiki seaweed (*Sargassum fusiforme*) because it contains high level of iAs.[10] Since Japanese people consume relatively higher amounts of seafood (fish, crustaceans, cephalopods, and mollusks) than other countries,[11] combined with rice and seaweed as key parts of their diet, it exposes them to the potentially higher risk of chronic As exposure.

Arsenobetaine (AsBe) is an organic As predominant in seafood, while seaweed mostly contains As in the form of arsenosugars.[12] Some seafood also contains arsenosugars and arsenolipids, which are mainly metabolized into dimethylarsinic acid (DMA ; Figure 1).[13, 14] Ingested AsBe is excreted unchanged in the urine and is generally considered to be a non-toxic arsenic species. However, arsenosugars have been shown to be cytotoxic *in vitro*, and the trivalent form of arsenosugars was more toxic than pentavalent ones.[15] Low levels of iAs are found in seafood as trivalent arsenite (As^{III}) and pentavalent arsenate (As^{V}). Still, certain mussels[16] and algae (most notably hijiki) may contain markedly higher levels of iAs.[5] Ingested iAs is rapidly absorbed from the blood and biomethylated in the liver, resulting in monomethylarsonic acid (MMA) and DMA, which can be readily excreted in the urine.[17] Urinary As levels are generally considered to reflect As exposure within the previous several days.[18]

iAs is classically thought to be detoxified by methylation into MMA and DMA, and this reaction may also be viewed as an activation mechanism because the trivalent intermediates in this pathway (MMA^{III} and DMA^{III}) exert stronger cytotoxicity than iAs.[19] Moreover, results obtained from experimental animal studies revealed DMA to be a carcinogen because it can be reduced into the toxic trivalent form DMA^{III} *in vivo*.[6] Therefore, the consumption of seafood and seaweed not only increases individual exposure to organic As, but also iAs, MMA, and DMA, with the associated health consequences, which are modulated to some extent by an individual capacity to metabolize As.

Genetic and environmental factors influence the capacity of an individual to metabolize arsenic and, hence, determine ingested As toxicity. Gender differences have been shown to play a role in arsenic metabolism in populations exposed to iAs from the drinking water, in which females methylate arsenic more efficiently than males.[20] Environmental factors known to modulate As metabolism include nutritional factors. Experimental evidence demonstrated that rabbits fed with choline-, methionine-, or protein-deficient diets showed decreased urinary excretion of As due to lower DMA excretion.[17] Dietary protein intake provides amino acids and B vitamins involved in the generation of S-adenosylmethionine (SAM), a methyl donor required

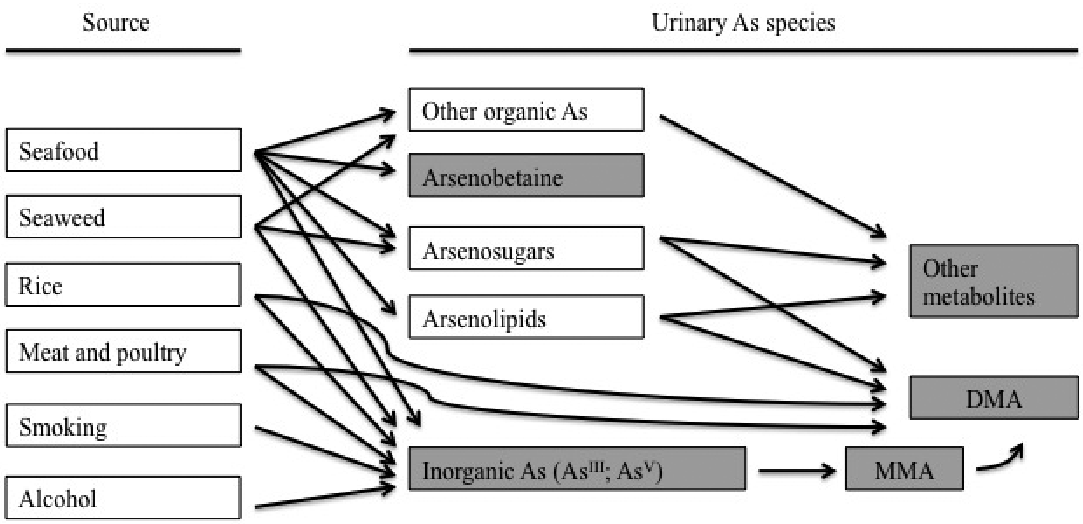

Figure 1. Contribution of As-containing foods, tobacco smoking, and alcohol to different As species excreted in the urine. As species detected in the urine were As^{III}, As^{V}, MMA, DMA, and arsenobetaine. Total urinary As minus the detected species was designated as other As metabolites.

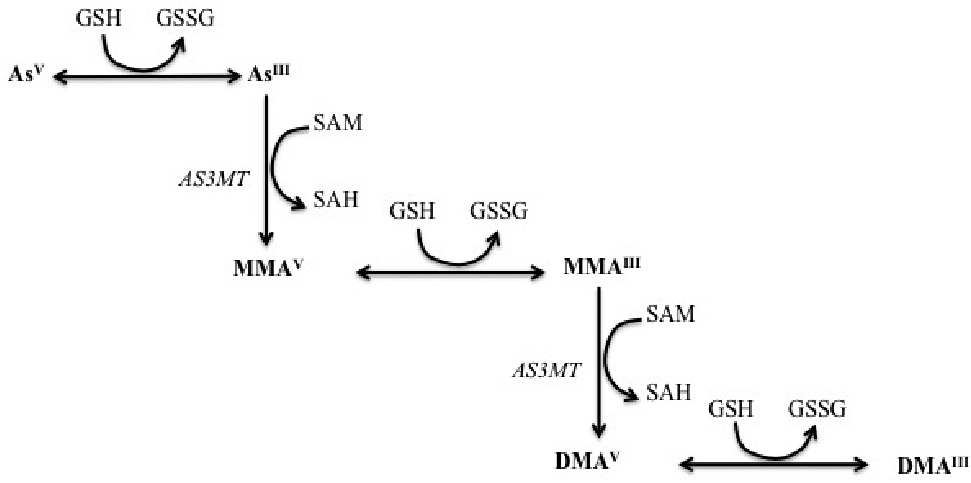

Figure 2. Inorganic arsenic biotransformation scheme in hepatocytes involving alternate steps of reduction from pentavalency to trivalency using glutathione (GSH) as the reductant, followed by the oxidative methylation of trivalent arsenicals using S-adenosylmethionine (SAM) as the methyl donor catalyzed by As^{III} methyltransferase (*AS3MT*). (Adapted from Hall *et al*, 2009).

for As methylation, which facilitates As excretion (Figure 2). Intakes of folate, riboflavin, niacin, thiamin, pyridoxine, and cobalamin were reported to modulate As metabolism in a Bangladeshi population chronically exposed to iAs.[21-23] Meanwhile, antioxidant vitamins (vitamin A, C, and E) have been shown to significantly modify the risk of arsenic-related skin lesions in the same population, in which malnutrition was prevalent.[22]

The present study examined urinary As excretion profiles and dietary factors associated with urinary As species in Japanese women from a coastal area to better understand dietary As exposure and the intake of nutrients potentially modulating As metabolism.

Materials and methods

Subjects

The data presented were derived from epidemiological studies conducted between 2006 and 2009, supported by a Grant-in-Aid on the Study Project on Heavy Metal-Containing Food from Food Safety Commission, Japan. Participants were female residents of fisherman families in Choshi, a coastal city in Chiba Prefecture, Japan, known to be high consumers of marine foods. Participation by female residents was voluntary. All participants completed a detailed questionnaire on lifestyle and dietary habits, including a semi-quantitative diet history questionnaire (DHQ), and underwent anthropometric measurements (height and weight). Participants also provided 2-hour urine samples.

The research protocol was approved by the Committee of Medical Ethics in Epidemiological Studies of Jichi Medical University and written informed consent was obtained from each participant before evaluations were initiated.

DHQ analysis

The self-administered DHQ used in this study has been described and validated previously.[24, 25] In brief, questions were designed to determine food and nutrient intake levels in the previous month with regard to portion size and semi-quantitative frequency of consumption of the 147 food items commonly consumed in Japan. DHQ contained 6 groups of rice, 7 groups of fish, fish products, cephalopods, and 3 groups of shellfish, roe, vegetables, fruits, milk, and soy products. In addition to DHQ, a supplementary questionnaire was also given to cover the consumption of frequently consumed fish species and sea algae, lacking in DHQ, to estimate a more precise intake of marine food items. It included 14 species of fish, 5 species of algae (wakame, nori, kombu, mozuku, and hijiki), 2 species of shellfish, whale meat, and the livers of 3 animal species. The intake of each food component was adjusted to daily energy requirements in order to avoid under-/over-reported bias and values based on 1000 kcal intake were used for statistical analyses. We considered that the estimated food intake in the previous month assessed using DHQ to be a reliable method to predict recent food intake, including seafood, as Navas-Acien et al (2011) demonstrated the frequency of seafood intake in the past year was related to the likelihood of recent seafood intake.[9]

Measures of urinary arsenic and creatinine

On arrival at the study sites, study participants were requested to empty their bladder and total urine generated in 2 hours was collected. Aliquots of specimens were collected in polypropylene tubes, sealed, and stored at -80℃ until analysis. Defrosted urine samples were diluted five-fold with ultrapure water, filtered through a 0.45 μm polyvinylidene fluoride membrane filter (Whatman, NJ, USA), and were analyzed by HPLC coupled with ICP-MS (Agilent Technologies, CA, USA) to separate and identify As species.[26] The species detected were trivalent inorganic As (As^{III}), pentavalent inorganic As (As^{V}), monomethylated (MMA^{V}) and dimethylated (DMA^{V}) metabolites, and arsenobetaine (AsBe). Other organic As species (Other As) took into account the sum of unknown peaks measured using the DMA standard in chromatograms. Unless otherwise stated, DMA and MMA in this study referred to DMA^{V} and MMA^{V}, respectively. Inorganic As referred to the sum of As^{III} and As^{V} because both species may undergo inter-conversion. Some analyses were performed using the proportion of urinary As species (i.e.%iAs, %MMA, %DMA) with iAs+MMA+DMA as the denominator.

Detection limits for As^{III}, As^{V}, MMA, DMA, and AsBe were 0.3 ; 0.2 ; 0.2 ; 0.3, and 0.4 μg/L, respectively. Concerning As^{III} and As^{V}, 19 and 2 samples were below the detection limits, respectively. The value of the detection limit divided by the square root of two was applied for non-detectable arsenic species. Assay accuracy was confirmed using reference material No.18 (human urine) from the National Institute for Environmental Studies (NIES), Tsukuba, Japan.[27] The laboratory performing As analysis adhered to quality assurance procedures and participated in an external quality program organized by the Institute of Occupational Social and Environmental Medicine of University of Erlangen, Nuremberg.

Urinary creatinine was analyzed spectrophotometrically using a commercial kit (Pure Auto CRE-N, Daiichi Pure Chemicals, Tokyo, Japan). Urinary As values were adjusted for dilution with urinary creatinine (i.e. μg/g creatinine). Unadjusted levels of As species were also calculated to facilitate comparisons with other studies, but were not presented in the results.

Considering the technical and logistic constraints, we did not check the reproducibility of the urine sample by consecutive sampling in the same subjects. However, a study on intra-individual variability of the urinary excretion of arsenic species in 10 consecutive days revealed no significant intra-individual variability in populations with a high intake of organic arsenic from dietary sources.[28]

Statistical analyses

Descriptive statistics were calculated for the general characteristics of the study sample. Because distributions of variables were non-normal, the nonparametric Spearman's correlation was used in bivariate analyses to examine associations between nutrient variables and urinary As variables. The statistical package used was IBM SPSS version 19.0 (IBM, Inc.). Values were considered significant at $p<0.05$.

Results

Subject characteristics

Study participants included 92 Japanese females from fisherman families with a mean age of 54.7 years (Table 1). The majority of subjects aged ≥50 years (67%) and body mass index (BMI) calculations based on a category specified for Asians (World Health Organization Western Pacific Regional Office (WPRO) criteria)[29] showed that nearly half of our subjects (47%) were obese. Most subjects were non-smokers and non-alcohol drinkers. Although total arsenic concentrations from the drinking water were not available, residents in the study area used a public water supply managed by the municipality with regulatory As levels of less than 10 μg/L.

Table 1
General characteristics of 92 women from a coastal area in Chiba Prefecture.

Age (years)		
Mean (SD)		54.7 (16.3)
Median		57.0
Range		14.0 - 81.0
BMI (n, (%))		
Underweight	(<18.5 kg/m^2)	3 (3)
Normal	(18.5-22.9 kg/m^2)	30 (33)
Overweight	(23.0-24.9 kg/m^2)	16 (17)
Obese	(>24.9 kg/m^2)	43 (47)
Smoking (n,(%))		
Never		78 (85)
Former		8 (9)
Current		6 (6)
Alcohol intake (n, (%))		
None		62 (67)
1-10 g/day		27 (30)
>10 g/day		3 (3)

SD = standard deviation; BMI = body mass index.

Urinary As metabolites

Geometric means, means, and medians of the detected urinary As species and urinary creatinine levels are listed in Table 2. The main urinary As metabolite was AsBe (163.4 μg/g ; geometric mean), followed by DMA (49.6 μg/g ; geometric mean) and other As (11.6 μg/g ; geometric mean). Meanwhile, iAs and MMA levels were 1.8 and 1.7 μg/g (geometric mean), respectively. To evaluate the amount of toxic As species in the urine, the sum of iAs, MMA, and DMA was calculated and used as a denominator to obtain the proportion of iAs (3.4 % ; geometric mean), MMA (3.2 % ; geometric mean), and DMA (91.6 % ; geometric mean).

Food and micronutrient intake

Daily intake of food and micronutrients was estimated from DHQ from each subject (Table 3). Based on the

Table 2

Concentration of urinary As species[a], proportion[b] of As metabolites, and urinary creatinine.

Variable	Geometric mean	Mean (SD)	Median (IQR)
iAs (μg/g)	1.8	2.4 (1.8)	1.9 (1.1-2.9)
MMA (μg/g)	1.7	1.4 (1.0)	1.7 (1.3-2.4)
DMA (μg/g)	49.6	42.7 (35.8)	55.1 (30.6-76.5)
AsBe (μg/g)	163.4	200.3 (325.5)	153.8 (89.5-375.2)
Other As (μg/g)	11.6	14.5 (18.7)	11.7 (5.5-27.3)
Total As (μg/g)	248.3	260.2 (352.8)	260.1 (144.7-445.6)
iAs+MMA+DMA (μg/g)	54.2	64.9 (39.3)	58.7 (35.2-85.6)
% iAs	3.4	4.6 (3.1)	3.8 (1.9-6.9)
% MMA	3.2	3.7 (2.3)	3.1 (2.1-4.9)
% DMA	91.6	91.7 (5.0)	93.1 (88.0-95.6)
Creatinine (mg/dL)	64.0	74.3 (42.6)	62.9 (45.9-93.6)

[a] *Adjusted with urinary creatinine.*

[b] *iAs+MMA+DMA as the denominator.*

SD = standard deviation; IQR = interquartile range.

Table 3

Estimated food groups, micronutrient, isoflavone, and soy protein daily intakes.

Variable	Daily intake	
	Mean (SD)	Median (IQR)
Seafood (g/1000 kcal)	49.0 (26.9)	44.2 (27.8-62.3)
Seaweed (g/1000 kcal)	5.5 (4.9)	3.5 (2.2-7.9)
Meat and poultry (g/1000 kcal)	24.4 (15.3)	22.1 (11.5-33.6)
White rice (g/1000 kcal)	112.3 (72.5)	97.3 (64.5-150.5)
Vegetables (g/1000 kcal)	119.8 (70.1)	101.1 (72.2-157.2)
Riboflavin (mg/1000 kcal)	0.7 (0.2)	0.7 (0.6-0.8)
Pyridoxine (mg/1000 kcal)	0.6 (0.2)	0.6 (0.5-0.6)
Folate (μg/1000 kcal)	158.3 (78.3)	151.4 (114.4-179.1)
Cobalamin (μg/1000 kcal)	4.3 (2.5)	3.7 (2.7-5.3)
Niacin (mg/1000 kcal)	8.3 (2.4)	7.9 (6.7-9.6)
Vitamin A (mg/1000 kcal)	3.7 (2.1)	3.3 (2.4-4.3)
Vitamin C (mg/1000 kcal)	70.8 (41.8)	64.7 (45.1-84.5)
Vitamin E (mg/1000 kcal)	11.4 (4.4)	10.7 (8.7-13.2)
Daidzein (mg/1000 kcal)	5.0 (2.8)	4.5 (3.1-6.6)
Genistein (mg/1000 kcal)	8.3 (4.7)	7.5 (5.1-10.9)
Soy protein (g/1000 kcal)	2.5 (1.5)	2.0 (1.5-3.3)

SD = standard deviation; IQR = interquartile range.

literature, the main food groups relevant to As exposure were seafood, seaweed, meat and poultry, white rice, and vegetables.[1, 2, 4] Total seafood intake was 44.2 g/1000 kcal/day (median) and total seaweed intake was 3.5 g/1000 kcal/day (median). In a separate analysis for detailed seaweed intake, we found that the average consumption of hijiki was 0.7 g/1000 kcal/day (data not shown).

The daily intake of several B vitamins, vitamin A, vitamin C, and vitamin E were estimated because several studies previously showed an association between these micronutrients and As metabolism, and also with As exposure profiles in people exposed to As from the drinking water.[21-23] Considering the distinctive diet of Japanese people due to the frequent consumption of soy-derived products, we also analyzed the intake of soy isoflavones (daidzein and genistein) and soy protein (Table 3).

Factors associated with urinary arsenic proportion

To examine factors associated with the proportion of toxic urinary As species (%iAs, %MMA, %DMA), bivariate analyses were performed using Spearman's correlation (Table 4). Age, BMI, and cigarette smoking were found to significantly correlate with urinary As variables. In the food group analysis, significant negative correlations were found for seafood and seaweed intake with %iAs (both $p<0.01$) and %MMA ($p<0.05$ for seafood ; $p<0.01$ for seaweed), and positive correlations with %DMA (both $p<0.01$). Meanwhile, meat and poultry intake showed a significant positive correlation ($p<0.05$) with %iAs, but a negative correlation with %DMA ($p<0.05$). No significant correlations were found for white rice and vegetable intake with urinary As variables.

In the micronutrient analysis, B vitamins generally showed significant correlations with urinary As proportion ($p<0.01$ for pyridoxine, $p<0.05$ for other B vitamins) (Table 4). Interestingly, all correlations with %iAs and %MMA were negative and all correlations with %DMA were positive. Vitamin C intake was negatively correlated with %

Table 4

Spearman's correlation coefficients showing factors associated with the proportion[a] of urinary As[b].

Variable	% iAs	% MMA	% DMA
Age (year)	-0.37**	-0.34**	0.39**
BMI (kg/m^2)	-0.27**	-0.22*	0.27**
Smoking (never,former,current)	0.24*	0.20	-0.24*
Possible dietary exposure source			
Seafood (g/1000 kcal)	-0.34**	-0.25*	0.33**
Seaweed (g/1000 kcal)	-0.36**	-0.28**	0.37**
Meat and poultry (g/1000 kcal)	0.22*	0.13	-0.21*
White rice (g/1000 kcal)	0.12	0.15	-0.16
Vegetable (g/1000 kcal)	-0.15	-0.20	0.19
Possible As metabolism modifier			
Riboflavin (mg/1000 kcal)	-0.19	-0.24*	0.22*
Pyridoxine (mg/1000 kcal)	-0.30**	-0.22*	0.30**
Folate (µg/1000 kcal)	-0.25*	-0.19	0.25*
Cobalamin (µg/1000 kcal)	-0.27*	-0.17	0.26*
Niacin (mg/1000 kcal)	-0.21*	-0.22*	0.24*
Vitamin A (mg/1000 kcal)	0.06	-0.08	0.03
Vitamin C (mg/1000 kcal)	-0.29**	-0.18	0.27**
Vitamin E (mg/1000 kcal)	-0.03	0.06	-0.00
Daidzein (mg/1000 kcal)	-0.23*	-0.31**	0.27**
Genistein (mg/1000 kcal)	-0.23*	-0.31**	0.27**
Soy protein (g/1000 kcal)	-0.24*	-0.29**	0.28**
Urinary As species			
AsBe (µg/g)	-0.65**	-0.51**	0.64**
Other As (µg/g)	-0.62**	-0.61**	0.66**
iAs+MMA+DMA (µg/g)	-0.62**	-0.64**	0.66**

[a] *iAs+MMA+DMA as the denominator.*

[b] *Adjusted with urinary creatinine.*

**p<0.05. **p<0.01.*

iAs (p<0.01) and positively correlated with %DMA (p<0.01), while no significant correlation was seen for vitamin A and E with urinary As proportions. Soy isoflavones and soy protein were negatively correlated with %iAs (p<0.05) and %MMA (p<0.01), but positively correlated with %DMA (p<0.01).

Furthermore, we also examined the association between urinary AsBe (the proxy of recent seafood intake), Other As (to account for the contribution of arsenosugars and arsenolipids to urinary DMA), and the sum of toxicologically relevant As species (as a measure of exposure burden) with the proportion of urinary As. Moderate correlations were observed between AsBe, other As, and the sum of toxic As species with the proportion of urinary iAs, MMA, and DMA (p<0.01). Correlations were negative with %iAs and % MMA, but positive with %DMA (Table 4).

Discussion

This study examined the urinary As excretion profiles of and associated dietary factors in Japanese women from a coastal area. The characteristics of our study population were of particular interest because the majority of participants were in the range of a postmenopausal age and nearly half of them were obese, which reflected their high nutritional status.

After examining urinary As excretion profiles (Table 2), we decided to further investigate whether the intake of major food groups, certain vitamins and nutrients involved in one-carbon metabolism (such as B vitamins and soy isoflavones) or reduction-oxidation reactions (vitamins A, C, E), were related to iAs metabolism (i.e. reduction and methylation) in populations exposed to As mostly from seafood and seaweed (Table 4).

To assess this possibility, we used the relative value of toxicologically relevant arsenic species (% iAs, %MMA, % DMA) rather than their absolute values. Using proportions was more appropriate in this case because some iAs may have been methylated into MMA and some MMA may have been methylated into DMA. The efficiency of the methylation process (which may have been influenced by some dietary factors mentioned above) should be reflected in the relative amount of iAs, MMA, and DMA. Previous studies showed that lower %iAs and %MMA, and higher % DMA were favorable because DMA is readily excreted in the urine.[20, 43, 44] Identifying an association between certain dietary factors and the proportion of As (rather than the absolute arsenic value) should provide information on the efficiency of the methylation process.

However, there is a caveat in this way of analysis. The observed correlation between nutrient intake and %DMA may be confounded given that the majority of urinary DMA in our subjects may have been derived from the metabolism of arsenosugars (from seaweed consumption) and arsenolipids (from fatty fish consumption), with only a minority coming from the methylation of iAs. Regression analysis between dietary factors and %DMA that includes other As variables as confounding factors is needed to resolve this issue, preferably with a larger population to obtain adequate statistical power.

Our subjects consumed seafood, seaweed, and rice on a daily basis. Our results suggest that seafood and seaweed intakes are the major determinants of the proportion of urinary DMA. Seafood contains arsenosugars and arsenolipids, which are metabolized into DMA.[14] Besides containing high levels of organoarsenicals, seafood and seaweed also contain iAs in varying amounts.[30] DMA may also be derived from the methylation of iAs; however, the contribution of iAs to urinary DMA in our subjects was unlikely to be significant. Seafood consumption studies in humans showed that DMA accounted for 1 – 42% of total As excreted in the urine, depending on the species of seafood consumed.[31, 32] Consistent with previous results and using total urinary As as the denominator, DMA accounted for 21% of excreted As in all subjects in our study. Although DMA from exogenous sources has been shown to be excreted mainly unchanged in human urine,[33] it is plausible that a small amount of DMA undergoes reduction to the toxic trivalent form based on findings in which DMA^{III} was present in the urine of humans exposed to iAs.[34] The carcinogenic effect of DMA through the formation of intermediate DMA^{III} has been evaluated in rats, known to be the most susceptible species to As toxicity.[6] However, it is unlikely that humans could generate cytotoxic level of DMA^{III} at the anticipated level of human exposure.[35]

As leading consumers of marine products in the world, the Japanese intake of iAs from foods was only 26% of the provisional tolerable weekly intake (PTWI).[3] The daily dietary intake of iAs (median 3.8 μg/day) in a small subset of Japanese women was estimated previously using duplicated diet samples.[36] In specific subjects with relatively high marine food consumption, such as ours, it is reasonable to expect a higher intake of iAs. However, using urinary iAs, which reliably reflects internal exposure, we found a slightly smaller value (median 1.85, IQR 1.05-2.85 μg/g). This result suggests that seafood consumption was not a risk factor for increased iAs exposure in our subjects.

We calculated the intake of several sea algae including hijiki to account for iAs exposure from seaweed. According to the 2008 National Diet Study, the average daily intake of sea vegetables in Japanese females aged 50-69 years was shown to be 11.3g.[37] Hijiki accounted for 6.1% of sea vegetable intake and its daily intake was estimated to be 0.7 g/2000 kcal/day, which is half of our estimation in this study (0.7 g/1000 kcal/day). When hijiki intake was incorporated into total seaweed intake and examined in bivariate analysis (Table 4), a significant negative correlation was seen with urinary %iAs. This result suggests that hijiki consumption, at least, was not a contributor to the increased As exposure in our population.

Rice can accumulate iAs from the soil; therefore, a rice-based diet may influence individual As exposure.[38, 39] An evaluation of rice intake is an important part of As exposure and risk assessment. However, the present study did not find any association between white rice intake and urinary As variables.

Interestingly, we demonstrated that meat and poultry intake was the only dietary factor positively correlated with %iAs in our subjects, albeit marginally. A recent study found that chicken meat in the U.S. contains iAs, DMA, and other As metabolites due to the use of As-based drugs to improve chicken growth.[40] Other than deliberate exposure through As-based drugs, chickens may be environmentally exposed to iAs from the drinking water, seafood-based meal, or soil. The relevance of this iAs exposure in our population seems to be negligible for a number of reasons. First, the use of As-based drugs is prohibited in the poultry industry in Japan. Second, the correlation seen was marginally significant, which indicated that this could be a chance finding that may diminish upon adjustments with other factors. Third, urinary iAs levels in our subjects were relatively low for such exposure to be of concern.

Total creatinine adjusted urinary As in our study population (mean: 260.2 μg/g) was similar to the urinary As levels of female high seafood consumers in France (mean: 265 μg/g),[41] but was higher than that of Korean ethnicity in the U.S. (mean: 152.1 μg/g).[42] However, the level of urinary iAs in our study (mean: 2.4 μg/g) was much lower than the same French study (mean: 27.1 μg/g) and slightly lower than the Korean ethnicity study (mean: 2.9 μg/g). The average value of the unadjusted sum of urinary As species (iAs, MMA, and DMA) in Japanese women in this study (≈ 40 μg/L; data not shown) was higher than that in the Korean women study in the U.S. (≈ 30 μg/L).[42]

B vitamins are involved in one-carbon metabolism to generate the universal methyl donor, SAM, which is essential for As methylation and excretion. Studies in a Bangladeshi population chronically exposed to high levels of As from drinking contaminated ground water demonstrated that the dietary intake of B vitamins related to individual capacity to methylate and excrete As from the body.[23, 43, 44] Moreover, B vitamins and antioxidant vitamin (vitamin A, C, and E) intakes were observed to modify the risk of arsenic-related skin lesions in the same population, in which malnutrition was prevalent.[22] In this study with well-nourished subjects, we also observed significant correlations for B vitamins and vitamin C intakes with %iAs, %MMA, and %DMA in the urine. This correlation was particularly obvious for pyridoxine. Other than the possibility that these vitamins influence the urinary As proportion, the positive correlations between vitamins and %DMA may reflect vitamins and DMA being derived from the same dietary source, such as seafood and seaweed.

A characteristic of the Japanese diet is the inclusion of soybeans and soy-based products, such as tofu, soy sauce, *natto* or fermented soybean, and *miso* or soybean paste in daily meals. It is possible that the dietary intake of isoflavones may have influenced the As metabolism capacity in a given individual. To investigate this potential link, we used DHQ that included soy foods and adjusted daily intakes of soy protein and isoflavones (daidzein and genistein) were calculated. Soy isoflavones and soy protein correlated negatively with %iAs and %MMA, but positively with %DMA in the urine. Our results suggest that soy intake marginally influences As metabolism. The average soy protein intake in this study (4.9 g/day, on assumption that the energy requirement is 2000 kcal/day) was lower than the Japanese national average (6.5 g/day).[45] Genistein and daidzein median intakes in this study (15 and 9 mg/day, respectively) were also lower than a previous report in middle-aged Japanese women (29.7 and 20.6 mg/day, respectively).[46]

The strengths of this study are that our subjects were fairly homogenous and urinary As metabolites were determined by the sensitive HPLC-ICP-MS method. Moreover, our validated DHQ covered extensive seafood and other food items with non-missing dietary data. However, the limitation of this study includes the cross-sectional nature of data collection. Furthermore, the number of subjects was small, which may have undermined the association between dietary factors and urinary As variables.

In conclusion, our results suggest that, despite the predominant contribution of seafood and seaweed to total urinary As, this population of Japanese women were exposed to low levels of iAs. We also demonstrated a correlation between the intake of B vitamins and vitamin C, but not vitamin A or vitamin E, and the proportion of urinary As species. We revealed for the first time a correlation between soy isoflavones intake and the proportion of urinary As species.

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Ethical approval : The research project was approved by the Committee of Medical Ethics in Epidemiological Studies (Approval No. Epi-06-16) of Jichi Medical University, Tochigi, Japan.

Conflict of Interest Statement

All the authors state that they have no conflicts of interest.

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漁協関係女性における尿中ヒ素化合物パターンへの食事の影響評価

Cimi Ilmiawati[1, 2], 小川　真規[1], 佐々木　敏[3], 渡邊　智子[4], 香山不二雄[1]

[1]自治医科大学医学部環境予防医学 , 〒320-0498 栃木県下野市薬師寺3311-1
[2]Department of Pharmacology, Faculty of Medicine, Andalas University, West Sumatra, Indonesia
[3]東京大学大学院医学研究科公共健康医学専攻疫学保健学講座社会予防疫学分野
[4]千葉県立保健医療大学健康科学部栄養学科

抄　　録

海産食品からのヒ素曝露が高い集団の尿中ヒ素排泄に関わる食品摂取の影響評価を目的として，漁協関係女性被験者92名の自記式食事調査票による栄養素摂取量と尿中ヒ素化合物排泄量をHPLC-ICP-MS 法にて測定した。尿中排泄量の幾何平均は，総ヒ素が248.3 μg/g cre，無機ヒ素は1.8 μg/g creであった。アルセノベタインやその他の毒性のないヒ素化合物を除いた有毒性ヒ素化合物の尿中比率は，無機ヒ素（iAs）3.4%，モノメチルアルソン酸（MMA）3.1%, ジメチルアルシン酸（DMA）91.6%であり，スピアマンの相関係数は，魚介類および海藻の摂取量と% iAs（r=-0.34; r=-0.36），%MMA（r=-0.25; r=-0.28），% DMA（r=0.33；r=0.37）とは，それぞれ有意の相関関係を示した。ビタミンB, C, 大豆イソフラボン摂取量が，iAs%，MMA%と負の相関，DMA%と正の相関を示した。結論として，調査集団の無機ヒ素曝露量は低く，ビタミンBおよびC摂取量は尿中ヒ素化合物比に相関があることを示した。また，大豆イソフラボン摂取量が尿中ヒ素化合物比に影響があることを初めて示した。

（キーワード：ヒ素曝露，尿中ヒ素代謝物，海産食品，日本人女性）

連絡先：香山不二雄，自治医科大学医学部環境予防医学，〒329-0498 栃木県下野市薬師寺3311-1，E-mail：kayamaf@jichi.ac.jp
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