ELSEVIER

Toxicology Letters 133 (2002) 83–91

Toxicology Letters

www.elsevier.com/locate/toxlet

Urinary arsenic speciation in subjects with or without restriction from seafood dietary intake

Yu-Mei Hsueh [a,*], Miao-Kan Hsu [a], Hung-Yi Chiou [b], Mo-Hsiung Yang [c], Chuan-Chieh Huang [c], Chien-Jen Chen [d]

[a] *Department of Public Health, School of Medicine, Taipei Medical University, No. 250, Wu Hsin Street, 110 Taipei, Taiwan, ROC*

[b] *School of Public Health, Taipei Medical University, Taipei, Taiwan, ROC*

[c] *Department of Nuclear Science, National Tsing-Hua University, Hsinchu, Taiwan, ROC*

[d] *Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan, ROC*

Abstract

In order to understand whether ingestion of seafood affects the urinary arsenic metabolites. About 42 women and 36 men were recruited from the students, parents and teachers in Taipei Medical University and National Taiwan University. The study subjects were interviewed about dietary habits, cigarette smoking habits, drug and vitamin intake, and consumption of seafood. Urine samples were collected from study subjects before and after refraining from eating seafood for 3 days, respectively. The urine samples were frozen at −20 °C separated by high-performance liquid chromatography (HPLC), and on line linked to hydride generator atomic absorption spectrometry (HGAAS) to quantify the levels of various species of inorganic arsenic and its metabolites. The levels of arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), total inorganic arsenic metabolites, inorganic arsenic percent, MMA percent and DMA percent were similar before and after refraining from eating seafood for 3 days. The frequencies of fish, shellfish and seaweed dietary intake were not significantly correlated with urinary arsenic species.

Keywords: Arsenic; Urinary arsenic speciation; Seafood; Dietary habits

1. Introduction

The fact that inorganic arsenic in the drinking water of millions of people exceeds maximal contamination level has become a major public health problem in the world (Abernathy et al., 1997). Arsenic has been classified as a human carcinogen to skin and lungs (World Health Organization, 1980). Epidemiological studies demonstrated a significant dose-response relationship among the long-term exposure to inorganic arsenic in drinking water and the mortality from cancers of the skin, lung, liver, bladder, kidney and prostate as well as various vascular diseases (Chen et al., 1988, 1992, 1995, 1996).

Accurate estimation of exposure is critical for a better understanding of the dose–response rela-

* Corresponding author.
E-mail address: ymhsueh@tmu.edu.tw (Y.-M. Hsueh).

0378-4274/02/$ - see front matter
PII: S0378-4274(02)00087-5

tionship between adverse health effects and inorganic arsenic exposure. Most epidemiological studies relied on measurements of arsenic in drinking water to estimate the exposure. Urinary excretion is the major pathway for the elimination of arsenic from the body (Crecelius, 1977). Chemical analysis of urine samples is a convenient approach to study the metabolism of arsenic compounds. Estimates of exposure to arsenic may be improved by the quantification of arsenic excreted in the urine.

The biotransformation process of inorganic arsenic in humans is very complicated. A substantial fraction of absorbed AsV is reduced in the blood to AsIII (Vahter and Envall, 1983; Marafante et al., 1985; Vahter and Marafante, 1985), which is then taken up by hepatocytes (Lerman et al., 1983) and methylated to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Thompson, 1993). The methylation may be considered a detoxification mechanism, because the methylated metabolites, in comparison with inorganic arsenic, are less reactive with tissue constituents (Tam et al., 1978), less toxic, and more readily excreted in the urine (Vahter, 1986, 1988). In general, inorganic arsenic and its metabolites in human urine contain 10–15% inorganic arsenic, 10–15% MMA, and 60–80% DMA (Buchet et al., 1981; Foa et al., 1984). However, recent studies suggest that methylated arsenic species, especially those in the trivalent state, may be more toxic than the present inorganic arsenic compounds (Styblo et al., 1997, 2000; Lin et al., 1999; Petrick et al., 2000). Properties that MMA (III) and DMA (III) are known to possess in various experimental systems include enzyme inhibition (Styblo et al., 1997; Lin et al., 1999) cell toxicology (Petrick et al., 2000), genotoxicity, and clastogenicity (Mass et al., 2001).

Consumption of seafood may result in a significant intake of arsenic because seafood contains high arsenic concentrations, generally in the form of organoarsenicals such as arsenobetaine and arsenosugars (Cullen and Reimer, 1989). Arsenobetaine ingested upon consumption of crab, lobster, shrimp, and fish is excreted rapidly into the urine unchanged (Le et al., 1993), and no toxic effect has been observed (Kaise and Fukui, 1992). It is thus difficult to use the total urinary arsenic concentration without speciating inorganic and organic arsenic as a biomarker of exposure to inorganic arsenic, unless the subjects refrain completely from eating seafood for sometime before urine samples are collected. A more suitable method for measuring exposure to inorganic arsenic is to determine inorganic arsenic and its metabolites using HPLC with a hydride generation system in combination with atomic absorption spectrometry (HGAAS) (Hakala and Pyy, 1992). This method is not influenced by the presence of arsenobetaine and arsenocholine in urine. The metabolism of arsenosugars is not well understood and the effect of arsenosugar ingestion on urinary arsenic excretion is not widely recognized. But, it has been shown that the ingestion of arsenosugar in seaweed may give rise to DMA in the urine (Ma and Le, 1998). It is important to understand whether ingestion of seafood affects the urinary level of inorganic arsenic metabolites in Taiwanese who are drinking tap water. For this reason, we use HPLC–HGAAS to determine the species of arsenic compounds in the urine of human subjects before and after refraining from eating seafood for 3 days. We also investigated other factors involved in the urinary arsenic metabolites.

2. Materials and methods

2.1. Study subjects

There were 78 healthy study subjects aged 20–61 years included in this study. The mean and standard error (S.E.) of urinary metabolite and fish, shellfish and seaweed intake frequency by age and sex distributions of study subjects in normal food intake are shown in Table 1. About 42 women and 36 men who were drinking tap water were recruited from the students, parents and teachers in Taipei Medical University and National Taiwan University. The study subjects were interviewed about dietary habits, cigarette smoking habits, drug and vitamin intake, and consumption of seafood. The total frequency of fish, shellfish and seaweed were calculated by fre-

Table 1
Mean and S.E. of urinary metabolite and fish, shellfish and seaweed intake frequency by age and sex distribution of study subjects in daily food intake

Age	Number	Total inorganic arsenic metabolites[a] (Mean ± S.E.)	Inorganic arsenic percent (Mean ± S.E.)	DMA percent (Mean ± S.E.)	MMA percent (Mean ± S.E.)	Fish intake frequency [b] (Mean ± S.E.)	Shellfish intake frequency [b] (Mean ± S.E.)	Seaweed intake frequency [b] (Mean ± S.E.)
Male								
20–29	18	52.8 ± 10.6	19.5 ± 1.8	62.1 ± 4.0	18.4 ± 3.4	124.2 ± 24.6*	63.6 ± 15.5	20.2 ± 8.6
30–39	9	52.9 ± 14.2	19.4 ± 2.9	67.7 ± 3.9	12.9 ± 3.4	253.5 ± 72.5	58.5 ± 24.9	63.6 ± 18.9
40–49	4	77.7 ± 38.6	23.6 ± 6.3	63.1 ± 7.6	13.3 ± 2.9	195.0 ± 109.0	65.0 ± 24.9	78.0 ± 33.6
≥50	5	62.9 ± 15.0	21.1 ± 5.2	60.5 ± 11.0	18.4 ± 6.9	468.0 ± 192.5*	62.4 ± 30.3	41.6 ± 30.3
Subtotal	36	57.0 ± 7.6	20.2 ± 1.5	63.7 ± 2.7	16.4 ± 2.1	211.0 ± 39.3	62.4 ± 10.6	40.4 ± 8.8
Female[c]								
20–29	21	48.5 ± 6.8	25.5 ± 2.1	54.0 ± 3.7	20.6 ± 2.5	141.8 ± 38.7	87.5 ± 17.2	92.2 ± 19.3
30–39	10	79.1 ± 17.8	18.6 ± 2.8	67.0 ± 5.7	14.4 ± 3.8	166.4 ± 37.8	67.6 ± 19.1	15.6 ± 11.1
40–49	5	81.6 ± 30.6	20.1 ± 2.2	62.3 ± 5.5	17.6 ± 3.9	303.3 ± 134.5	60.7 ± 16.0	17.3 ± 10.9
≥50	4	127.7 ± 55.7	16.8 ± 3.7	73.6 ± 6.5	9.6 ± 3.0	364.0 ± 182.6	52.0 ± 21.2	78.0 ± 33.6
Subtotal	40	68.2 ± 9.1	22.2 ± 1.5	60.2 ± 2.8	17.6 ± 1.8	187.7 ± 33.0	75.5 ± 10.5	61.9 ± 12.4
Total	76	62.9 ± 6.0	21.3 ± 1.0	61.7 ± 1.9	17.0 ± 1.4	198.4 ± 25.3	69.6 ± 7.5	52.01 ± 7.9

Total inorganic arsenic metabolites = AsIII + AsV + MMA + DMA; Inorganic arsenic% = (AsIII + AsV)/total inorganic arsenic metabolites) × 100; MMA% = (MMA/total inorganic arsenic metabolites) × 100; DMA% = (DMA/total inorganic arsenic metabolites) × 100. *$P < 0.05$

[a] mg/g creatinine.

[b] Frequency per week × 52 weeks.

[c] Two females did not have urine.

quency per week multiplied 52 weeks in daily food intake. The 12-h overnight urine samples were collected from recruited study subjects before and after refraining from eating seafood for 3 days and stored at -20 °C until analysis.

2.2. Arsenic measurements

The urine samples were tested for levels of AsIII, AsV, MMA and DMA. To achieve a more accurate assessment of arsenic methylation capability, it is necessary to determine specifically only those arsenic species derived from inorganic arsenic and excreted in the urine. Urine samples were thawed at room temperature, dispersed by ultrasonic wave, and filtered through Sep-Pak C_{18} column. Arsenic species in 200 μl urine were separated using a HPLC system (Hitachi 6000, Japan) equipped with a HPLC column (Phenomenex, Nucleosil 10SB, 100A, Torrance, CA, USA), and linked to a HGAAS (Perkin Element) for the quantification (Hakala and Pyy, 1992). Total arsenic in urine was defined as the sum of AsIII, AsV, MMA and DMA. Recovery rates for AsIII, DMA, MMA and AsV were 96.9 ± 0.2, 93.8 ± 0.7, 102.2 ± 0.5 and $94.1 \pm 0.2\%$, respectively, with the detection limits of 0.2, 0.6, 0.7 and 0.1 μg/l, respectively. Freeze-dried urine SRM 2670, containing 480 ± 100 μg/l arsenic, was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA) and analyzed together with test urine samples to control for the quality of the method. A standard value of 507 ± 17 μg/l ($n = 4$) was recorded.

2.3. Data analysis

In addition to the levels of inorganic and organic arsenic in urines, the percentages of AsIII, AsV, MMA and DMA in total arsenic were also analyzed to examine the arsenic methylation capability of study subjects. An increased percentage of AsIII + AsV in total arsenic and/or a decreased percentage of DMA in total arsenic reflect a decreased methylation capability. Mean and S.E. of urinary arsenic levels and percentages of various arsenic metabolites were calculated and analyzed by Student's t-test for different gender, and by ANOVA for differences in age groups. Paired t-test was used to compare various arsenic metabolites or percentages of various arsenic metabolites of study subjects between ingested seafood and refrained from eating seafood for 3 days by paired t-test. As age, and sex were all related to urinary levels or percentages of various arsenic metabolites, it was essential to examine the correlation between urinary arsenic levels and percentages of arsenic species for a given independent variable when other factors were adjusted through multivariate analysis. Multiple regression analysis was thus used to evaluate the association of urinary levels or percentages of various arsenic metabolites with age, sex and the consumption of seafood.

3. Results

Seventy-eight study subjects were initially recruited to participate in the study. Two subjects did not collect their normal diet urines and 13 subjects did not collect their urines who refrained from eating seafood for 3 days before the sample collection day. Females comprised more than half of the study subjects 53% (40/76). Approximately half of the subjects 51% (39/76) were 20–29 years of age.

The results of the mean and S.E. of urinary inorganic arsenic metabolite and intake frequency of fish, shellfish and seaweed by age and sex distribution of study subjects in daily food intake are presented in Table 1. The total inorganic arsenic metabolites, inorganic arsenic percent, MMA percent, and DMA percent of 76 study subjects were 62.9 ± 6.0 mg/g creatinine (29.91 ± 2.33 μg/l), 21.3 ± 1.0, 17.0 ± 1.4 and $61.7 \pm 1.9\%$, respectively. There was no significant difference among age groups for different gender. The total inorganic arsenic metabolites were lower than those of healthy residents from the blackfoot disease endemic area (86.08 ± 3.43 μg/l) (Hsueh et al., 1998), and those from Lanyang Basin (172.9 ± 18.6 μg/l) (Chiou et al., 1997), in Taiwan. We found that fish frequency had a significant difference between 20–29 and ≥ 50 age groups, but shellfish and seaweed frequency had no sig-

nificant differences among age groups for different gender.

Similar trends were observed for the total inorganic arsenic metabolites, inorganic arsenic percent, MMA percent and DMA percent before and after refraining from eating seafood for 3 days (Table 2). The average urinary total inorganic arsenic metabolites before and after refraining from eating seafood for 3 days were 62.89 ± 5.95 and 55.49 ± 7.44 mg/g creatinine, respectively. The effect of refraining from eating seafood on species distribution for all participants combined showed a mean increase of 0.86% (21.25–22.44%) and 0.81% (61.73–62.38%) in the inorganic arsenic percent and DMA percent, respectively. But there was a decrease of 1.68% (17.03–15.17%) in the MMA percent. Stratification of gender also showed same results for the total inorganic arsenic metabolites, inorganic arsenic percent, MMA percent and DMA percent. Stratified age groups for the comparison of arsenic metabolite species before and after refraining from eating seafood for 3 days showed no significant differences (Table 3).

When compared by cigarette smoking status, the MMA was higher for the smoking group before refraining from eating seafood than after refraining from eating seafood. The AsV concentration was higher for the non-smoking group than smoking group after refraining from eating seafood. The total arsenic concentrations were 64.23 ± 6.64, 51.55 ± 5.66, 53.28 ± 6.93, and 73.76 ± 40.6 mg/g creatinine for the non-smoking group, smoking group before refraining from eating seafood, and the non-smoking group, smoking group after refraining from eating seafood, respectively (Table 4). These four groups were not significantly different in the AsIII, DMA, total inorganic arsenic metabolites, inorganic arsenic percent, MMA percent and DMA percent. There was no significant difference between vegetarians and non-vegetarians in the total inorganic arsenic metabolites (53.47 ± 0.99 vs. 63.70 ± 0.64 mg/g creatinine), inorganic arsenic percent (22.63 ± 6.26 vs. $21.13 \pm 1.01\%$), MMA percent (16.67 ± 6.05 vs. $17.06 \pm 1.41\%$) and DMA percent (60.70 ± 9.00 vs. $61.81 \pm 1.98\%$).

The results of the regression analysis using urinary arsenic species as the dependent variables and fish, shellfish, and seaweed frequency as the independent variables are presented in Table 5. We found that fish, shellfish and seaweed frequency were not significantly correlated with the urinary arsenic species.

Table 2
Urinary arsenic metabolites distribution before and after refraining seafood 3 days by gender

Sex		Total inorganic arsenic metabolites (mg/g creatinine) (Mean ± S.E.)	Inorganic arsenic percent (Mean ± S.E.)	MMA percent (Mean ± S.E.)	DMA percent (Mean ± S.E.)
Male	Before (36)	57.01 ± 7.63	20.18 ± 1.45	16.43 ± 2.12	63.38 ± 2.72
	After (29)	53.117 ± 12.03	21.96 ± 3.07	16.27 ± 2.07	61.77 ± 3.50
	P value*	0.94	0.59	0.96	0.65
Female	Before (40)	68.19 ± 9.01	22.21 ± 1.47	17.56 ± 1.78	60.23 ± 2.75
	After (36)	57.40 ± 9.46	22.86 ± 2.82	14.22 ± 2.18	62.92 ± 3.37
	P value*	0.55	0.96	0.29	0.42
Total	Before (76)	62.89 ± 5.98	21.25 ± 1.03	17.03 ± 1.37	61.73 ± 1.94
	After (65)	55.49 ± 7.44	22.44 ± 2.06	15.17 ± 1.51	62.38 ± 2.41
	P value*	0.63	0.67	0.44	0.77

Paired *t*-test. Inorganic arsenic% = (AsIII + AsV)/total inorganic arsenic metabolites) × 100; MMA% = (MMA/total inorganic arsenic metabolites) × 100; DMA% = (DMA/total inorganic arsenic metabolites) × 100.

Table 3
Urinary arsenic metabolite distribution before and after refraining seafood 3 days stratified on age

Age (year)		Total inorganic arsenic metabolites (mg/g creatinine) (Mean ± S.E.)	Inorganic arsenic percent (Mean ± S.E.)	MMA percent (Mean ± S.E.)	DMA percent (Mean ± S.E.)
20–29	Before (39)	50.47 ± 6.02	22.74 ± 1.46	19.54 ± 2.06	57.72 ± 2.77
	After (33)	58.57 ± 12.07	27.16 ± 3.70	13.52 ± 2.21	59.32 ± 3.94
	P value*	0.51	0.24	0.06	0.67
30–39	Before (19)	66.70 ± 11.62	18.97 ± 1.98	13.69 ± 2.50	67.33 ± 3.41
	After (15)	55.91 ± 16.52	16.97 ± 2.26	19.96 ± 3.10	63.07 ± 3.88
	P value	0.67	0.38	0.13	0.28
40–49	Before (9)	79.88 ± 22.55	21.65 ± 2.86	15.70 ± 2.50	62.65 ± 4.24
	After (8)	53.15 ± 14.52	20.59 ± 3.68	12.02 ± 3.77	67.39 ± 6.46
	P value	0.16	0.63	0.50	0.51
50	Before (9)	91.70 ± 26.63	19.18 ± 3.23	14.50 ± 4.14	66.32 ± 6.79
	After (9)	45.53 ± 9.45	16.50 ± 2.92	16.08 ± 3.75	67.42 ± 4.98
	P value	0.13	0.50	0.75	0.87

Total inorganic arsenic metabolites = AsIII + AsV + MMA + DMA; Inorganic arsenic% = (AsIII + AsV)/total inorganic arsenic metabolites) × 100; MMA% = (MMA/total inorganic arsenic metabolites) × 100; DMA% = (DMA/total inorganic arsenic metabolites) × 100.

4. Discussion

Ingestion from food and water is the major route of exposure to arsenic by the general population (World Health Organization, 1981; Pontius et al., 1994). In the blackfoot disease endemic area, the average arsenic, predominantly AsIII, concentration in three wells was 671 ± 149 μg/l. The ratio of AsIII:AsV was 2.6:1 (Chen et al., 1994). It was reported that the AsIII and AsV percent in Taiwanese rice and yams extracted with water or hydrochloric acid followed by HPLC-ICP-MS for the speciation were 72%, and 76% extracted with hydrochloric acid and 69%, and 33% extracted with water respectively (Schoof et al., 1998). However, there are uncertainties about the relationship between exposure to arsenic and its clearance in the urine, which as a biomarker of exposure to arsenic in drinking water. The most commonly used biomarkers of exposure (or internal dose) to inorganic arsenic are based on the measurement of urinary AsIII, AsV, MMA, and DMA because urinary excretion is the main pathway for the elimination of arsenic. Recently, a study indicated that there was little day-to-day variation in the concentration of arsenic in urine (Hopenhayn-Rich et al., 1999). The concentration of arsenic in urine may not vary by gender, and an age-dependent difference in the concentration of arsenic in urine may be attributed to the higher arsenic dosage rate per unit body weight in children than in adults (Hopenhayn-Rich et al., 1999). We found that women had a significantly higher percentage of DMA in total arsenic and lower percentage of MMA in total arsenic than men after the adjustment for age and cumulative exposure to arsenic in the residents of the blackfoot disease endemic area (Hsueh et al., 1998). In addition, age effect on arsenic species in urine may reflect a poor methylation among the elderly (Hsueh et al., 1998).

Ingestion of arsenic from all sources contributes to the total urinary arsenic concentration. To obtain a reliable assessment of arsenic exposure from a particular source, e.g. drinking water, one must ensure that the ingestion of arsenic from other sources can be differentiated and confounding factors can be identified. Arsenic species from seafood sources are usually eliminated from the body within 3 days after ingestion (Le and Ma,

1997). For this reason, subjects should not eat any seafood for at least 3 days before urine samples are taken for the assessment of exposure to inorganic arsenic. In this study, all study subjects were volunteers and they were informed about the study aim and carefully refrained from seafood for 3 days prior to the urine collection.

This is the first study to investigate the effect of seafood consumption on urinary arsenic metabolites in Taiwanese who are drinking tap water. In addition, this research design has the advantage of having each person serve as his/her own control, thus reducing interindividual variability found when comparing two populations with contrasting exposures. In this study, the overall distributions of urinary arsenic metabolites of AsIII, AsV, MMA, DMA, and total inorganic arsenic metabolites were similar before and after intervention. Furthermore, gender and age did not confound the difference of urinary arsenic metabolites of AsIII, AsV, MMA, DMA, and total inorganic arsenic metabolites before and after intervention. No significant differences were observed in the levels of urinary arsenic metabolites between vegetarians and non-vegetarians. Our results demonstrated that the frequencies of fish, shellfish, and seaweed were not related with DMA and DMA percent. These results seem contradictory to those observed from Japanese volunteers after the consumption of seaweed (Ma and Le, 1998). Average daily consumption of seaweed by the Taiwanese might not have been as high as the Japanese.

The greatest changes in the secondary index occurred between non-smokers and smokers and the latter appeared to be less efficient methylators at the second methylation step (MMA to DMA), and the differences were similar to those reported in a previous cross-sectional study (Hopenhayn-Rich et al., 1996). It is possible that chemicals in cigarette smoke compete for some of the same detoxication enzymes or co-factors involving inorganic arsenic methylation, particularly in the second methylation step.

In summary, this study provided suggestive evidence that the levels of AsIII, AsV, MMA, DMA, total arsenic metabolites, inorganic arsenic percent, MMA percent and DMA percent before and after refraining from eating seafood for 3 days were similar in Taiwanese who are drinking tap water.

Table 4
Urinary arsenic metabolite distribution by cigarette smoking status

Variables	Before		After	
	No smoking	Smoking	No smoking	Smoking
	Mean ± S.E. (Number)	Mean ± S.E. (Number)	Mean ± S.E. (Number)	Mean ± S.E. (Number)
AsIII (mg/g creatinine)	4.69 ± 0.67(68)	3.75 ± 0.49(8)	3.42 ± 0.45(58)	5.47 ± 2.56(7)
AsV (mg/g creatinine)	9.85 ± 1.37(68)	7.29 ± 1.74(8)	7.54 ± 1.22[a](58)	2.31 ± 0.51[a](7)
MMA (mg/g creatinine)	11.38 ± 1.70(68)	8.89 ± 1.86[b](8)	8.47 ± 1.91(58)	6.86 ± 2.75[b](7)
DMA (mg/g creatinine)	38.30 ± 4.15(68)	31.61 ± 3.81(8)	33.85 ± 4.98(58)	59.10 ± 38.80 (7)
Total inorganic arsenic metabolites (mg/g creatinine)	64.23 ± 6.64(68)	51.55 ± 5.66(8)	53.28 ± 6.93(58)	73.76 ± 40.62(7)
Inorganic arsenic percent	21.34 ± 1.12(68)	20.43 ± 2.42(8)	22.71 ± 2.18(64)	19.98 ± 6.74 (7)
MMA percent	17.10 ± 1.48(68)	16.44 ± 3.49(8)	15.03 ± 1.60(64)	16.54 ± 4.78 (7)
DMA percent	61.56 ± 2.08(68)	63.13 ± 5.40(8)	62.26 ± 2.56(64)	63.48 ± 7.62 (7)

Total inorganic arsenic metabolites = AsIII + AsV + MMA + DMA; Inorganic arsenic % = (AsIII + AsV)/total inorganic arsenic metabolites × 100; MMA% = (MMA/total inorganic arsenic metabolites) × 100; DMA% = (DMA/total inorganic arsenic metabolites) × 100.

[a] The t-test for non smoking versus smoking, $P < 0.05$.

[b] Paired t-test, $P < 0.05$.

Table 5
Association between fish, shellfish and seaweed intake frequency and urinary arsenic metabolites in daily food intake

Variable	Fish frequency[a]		Shellfish frequency[a]		Seaweed frequency[a]	
	Regression coefficient[b]	Standard error	Regression coefficient	Standard error	Regression coefficient	Standard error
Arsenite (mg/g creatinine)	−0.08	0.3	−0.39	0.94	−0.38	0.88
Arsenate (mg/g creatinine)	−0.09	0.7	−1.04	1.95	−0.18	1.84
MMA (mg/g creatinine)	0.39	0.8	1.42	2.43	−3.22	2.20
DMA (mg/g creatinine)	−1.12	1.8	−10.6	5.0	−1.81	5.17
Total inorganic arsenic (mg/g creatinine)	−0.90	3.03	−10.6	8.98	−5.57	8.49

Total inorganic arsenic metabolites = AsIII + AsV + MMA + DMA.
[a] Frequency per week × 52 weeks.
[b] Age, sex adjusted regression coefficient × 10^{-3}.

Acknowledgements

This study was supported by grants from the National Science Council, Executive Yuan, Taiwan (NSC-88-2815-C-038-006-B; NSC-88-2314-B-038-112; NSC-88-2318-B-038-002-M51; NSC-89-2320-B-038-013; NSC-89-2318-B-038-002-M51; NSC-87-2314-B-029).

References

Abernathy, C.O., Calderon, R.L., Chappell, W., 1997. Arsenic: Exposure and Health Effects. Chapman & Hall, London.

Buchet, J.P., Lauwerys, R., Roels, H., 1981. Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. Int. Arch. Occup. Environ. Health 48, 71–79.

Chen, C.J., Kuo, T.L., Wu, M.M., 1988. Arsenic and cancers, Lancet, 414–415.

Chen, C.J., Chen, C.W., Wu, M.M., Kuo, T.L., 1992. Cancer potential in liver, lung, bladder, and kidney due to ingested inorganic arsenic in drinking water. Br. J. Cancer 66, 888–892.

Chen, S.L., Dzeng, S.R., Yang, M.H., Chiu, K.H., Shih, G.M., Wai, C.M., 1994. Arsenic species in ground water of the blackfoot disease area Taiwan. Environ. Sci. Technol. 28, 877–881.

Chen, C.J., Hsueh, Y.M., Lai, M.S., Shyu, M.P., Chen, S.Y., Wu, M.M., Kuo, T.L., Tai, T.Y., 1995. Increased prevalence of hypertension and long-term arsenic exposure. Hypertension 25, 53–60.

Chen, C.J., Chiou, H.Y., Chiang, M.H., Lin, L.J., Tai, T.Y., 1996. Dose-response relationship between ischemic heart disease mortality and long-term arsenic exposure. Arterioscler. Thromb. Vasc. Biol. 16, 504–510.

Chiou, H.Y., Hsueh, Y.M., Hsieh, L.L., Hsu, L.I, Hsu, Y.H., Hsieh, F.I, Wei, M.L., Chen, H.C., Yang, H.T., Leu, L.C., Chen, C.J., 1997. Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutat. Res. 386, 197–207.

Cullen, W.R, Reimer, K.J., 1989. Arsenic speciation in the environment. Chem. Rev. 89, 713–764.

Crecelius, E.A., 1977. Changes in the chemical speciation of arsenic following ingestion by man. Environ. Health Perspect. 19, 147–150.

Foa, K.V., Colombi, A., Maroni, M., Buratti, M., Calzaferri G., 1984. The speciation of the chemical forms of arsenic in the biological monitoring of exposure to inorganic. Sci. Total Environ. 34, 241–259.

Hakala, E., Pyy, L., 1992. Selective determination of toxicologically important arsenic species in urine by high-performance liquid chromatography-hydride generation atom absorption spectrometry. J. Anal. Atomic Spectro. 7, 191–196.

Hopenhayn-Rich, C., Biggs, M.L., Smith, A.H., Kalman, D.A., Moore, L.E., 1996. Methylation study of a population environmentally exposed to arsenic in drinking water. Environ. Health Perspect. 104, 620–628.

Hopenhayn-Rich, C., Hudgens, E., Le, X.C., Schreinemachers, D., Thomas, D., 1999. Excretion of arsenic in urine as a function of exposure to arsenic in drinking water. Environ. Health Perspect. 107, 663–667.

Hsueh, Y.M., Huang, Y.L., Huang, C.C., Wu, W.L., Chen, H.M., Yang, M.H., Lue, L.C., Chen, C.J., 1998. Urinary levels of inorganic and organic arsenic metabolites among residents in an arseniasis-hyperendemic area in Taiwan. J. Toxicol. Environ. Health 54, 431–444.

Kaise, T., Fukui, S., 1992. The chemical form and acute toxicity of arsenic compounds in marine organisms. Appl. Organomet. Chem. 6, 155–160.

Le, X.C., Ma, M., 1997. Speciation of arsenic compounds using ion pair chromatography with atomic spectrometry and mass spectrometry detection. J. Chromatogr. A 764, 55–64.

Le, X.C., Cullen, W.R., Reimer, K.J., 1993. Determination of urinary arsenic and impact of dietary arsenic intake. Talanta 40, 185–193.

Lerman, S.A., Clarkson, T.W., Gerson, R.J., 1983. Arsenic uptake and metabolism by liver cells is dependent on arsenic oxidation state. Chem. Biol. Interact. 45, 401–406.

Lin, S., Cullen, W.R., Thomas, D.J., 1999. Methylarsenicals and arsinothiols are potent inhibitors of mouse liver thioredoxin reductase. Chem. Res. Toxicol. 12, 924–930.

Marafante, E., Vahter, M., Envall, J., 1985. The role of the methylation in the detoxication of arsenate in the rabbit. Chem. Biol. Interact. 56, 225–238.

Ma, M., Le, X.C., 1998. Effect of arsenosugar ingestion on urinary arsenic specation. Clin. Chem. 44 (3), 539–550.

Mass, M.J., Tennant, A., Roop, B.C., Cullen, W.R., Styblo, M., Thomas, D.J., Kligerman, A.D., 2001. Methylated trivalent arsenic species are genotoxic. Chem. Res. Toxicol. 14 (4), 355–361.

Petrick, J.C., Ayala-Fierro, F., Cullen, W.R., Carter, D.C., Aposhian, H.V., 2000. Monomethyl arsonons acid (MMA) is more toxic than arsenite in Chang human hepatocytes. Toxicol. Appl. Pharm. 163, 203–207.

Pontius, F.W., Brown, K.G., Chen, C.J., 1994. Health implications of arsenic in drinking water. J. Am. Water Works Assoc. 86, 52–63.

Schoof, R.A., Yost, L.J., Crecelius, E., Irgolic, K., Goessler, W., Guo, H.R., Greene, H., 1998. Dietary arsenic intake in Taiwanese districts with elevated arsenic in drinking water. Hum. Ecol. Risk Assess 4, 117–135.

Styblo, M., Serves, S.V., Cullen, W.R., Thomas, D.J., 1997. Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. Chem. Res. Toxicol. 10, 27–33.

Styblo, M., Del Razo, L.M., Vega, L., Germolec, E.L., LeCluyse, E.L., Hamilton, G.A., Reed, W., Wang, C., Cullen, W.R., Thomas, D.J., 2000. Comparative toxicity of trivalent and pentavalent inorganic and methylaed arsenicals in rat and human cells. Arch. Toxicol. 74, 289–299.

Tam, K.H., Charbonneau, S.M., Bryce, F., Lacroix, G., 1978. Separation of arsenic metabolites in dog plasma and urine following intravenous injection of 74 As. Anal. Biochem. 86, 505–511.

Thompson, D.J., 1993. A chemical hypothesis for arsenic methylation in mammals. Chem. Biol. Interact. 88, 89–114.

Vahter, M., Envall, J., 1983. In vivo reduction of arsenate in mice and rabbits. Environ. Res. 32, 14–24.

Vahter, M., Marafante, E., 1985. Reduction and binding of arsenate in marmoset monkeys. Arch. Toxicol. 57, 119–124.

Vahter, M., 1986. Environmental and occupational exposure to inorganic arsenic. Acta Pharmacol. Toxicol. 59, 31–34.

Vahter, M., 1988. Arsenic. In: Clarkson, T.W., Friberg, L., Nordberg, G.F., Sager, P.R. (Eds.), Biological Monitoring of Toxic Metals. Plenum, New York, pp. 303–321.

World Health Organization, 1980. IARC. monographs on the evaluation of the carcinogenic risk of chemical to humans: Some metal and metallic compounds. pp.39–141. IARC Scientific Publ. No.23, Lyon, France: International Agency Research on Cancer.

World Health Organization, Environmental Health Criteria 18: Arsenic. World Health Organization, Geneva 1981.