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Determinants of inorganic arsenic methylation capability among residents of the Lanyang Basin, Taiwan: arsenic and selenium exposure and alcohol consumption

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Abstract

The objective of this study was to assess individual variation in inorganic arsenic methylation capability and the association between selenium levels in urine and blood, and inorganic arsenic methylation capability among residents of the Lanyang Basin who drank groundwater and were exposed to high concentrations of inorganic arsenic. According to the arsenic concentration of their drinking water, they were equally and randomly classified into four groups of 252 persons. It turned out that the higher the concentration of arsenic in well water was and thus the cumulative arsenic exposure, the higher the total inorganic arsenic metabolites in urine (total As_i) and the overall inorganic and organic arsenic in urine (overall As_{i+o}) were. The percentage of inorganic arsenic significantly decreased and the DMA percentage significantly increased as the concentration of urinary selenium and serum α-tocopherol increased. It appeared that urinary selenium levels increased the metabolism by methylation of arsenic, a finding that requires further investigation.

Keywords: Arsenic; Selenium; Micronutrient; Arsenic methylation

1. Introduction

Arsenic has been classified as a human carcinogen to the skin and lungs. Inhalation of arsenic mainly from occupational exposure such as in metal smelting and pesticide production increases the risk of lung cancer, while ingestion of arsenic, mainly from contaminated drinking water, causes skin cancer (IARC, 1980). Epidemiological studies have also demonstrated a significant dose–response relationship among long-term exposure to inorganic arsenic in drinking water and mortality from cancers of the skin, lung, liver, bladder, kidney, and prostate as well as lethality from

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various vascular diseases (Chen et al., 1988, 1992, 1995, 1996).

Inorganic arsenic as pentavalent arsenate (AsV) and trivalent arsenite (AsIII) are the major forms of arsenic in surface and groundwater (Korte and Fernando, 1991; Irgolic, 1994). Consumption of drinking water containing AsIII or AsV is the predominant source of exposure to inorganic arsenic worldwide. In blackfoot disease (BFD) endemic areas of Taiwan, the average arsenic, predominantly arsenite, concentration in three wells was 671 ± 149 μg/l. The ratio of AsIII to AsV was 2.6:1 (Chen et al., 1994). Outside the BFD area, the arsenic concentration dropped to 0.7 μg/l. When ingested in dissolved form, inorganic arsenic is readily absorbed. About 80–90% of a single dose of arsenite or arsenate was absorbed from the human gastrointestinal tract (Pomroy et al., 1980). The biotransformation processes of inorganic arsenic in human are 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 become 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; 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).

Selenium is an essential trace element with a recommended dietary allowance of 70 μg per day for adults (National Research Council Recommended Dietary Allowances, 1989). Selenium is an essential component of glutathione peroxidase, which plays a critical role in the body's antioxidant defense against the deleterious effects of free radicals (Ursini and Bindoli, 1987; Rayman, 2000). The majority of animal studies related to selenium and cancer have reported a protective effect of selenium including inhibition of carcinogenesis (Reddy et al., 1997). Prospective studies carried out among populations with a low or moderate selenium intake have found an inverse association between selenium levels in the blood or toenails and cancer at all sites (Knekt et al., 1990). The interaction of selenium with various metals produces protective effects in vitro and in vivo (Naganuma et al., 1983). Arsenic and selenium are metalloids with similar chemical properties but with markedly different biological effects. That selenium offers protection against arsenic toxicity is well known (Andersen and Nielsen, 1994), since the two metals act as metabolic antipodes (Schrauzer, 1992). Toxicological and metabolic interactions between arsenic and selenium have been widely reported in the literature. However, most investigations have focused on the influence of selenium on arsenic toxicity and its disposition in animal studies. These interactions are of interest from a public health standpoint because there are numerous areas throughout the world in which populations are exposed to relatively high levels of inorganic arsenic in drinking water together with varying levels of selenium in the diet. Urinary selenium was shown to reflect the total amount of selenium absorbed in any chemical form (Shiobara et al., 1998). The aim of this study was to evaluate whether differing urinary levels of selenium alter the disposition and methylation of orally administered inorganic arsenic from drinking water.

2. Materials and methods

2.1. Study area

In total, 18 villages in four townships of the Lanyang Basin located on the northeastern coast of Taiwan were included in the present study.

These areas included Yukuang, Baiyun, Kuangwu, and Yutien in Chiaohsi Township; Kunting, Junghsiau, Meichern, Jishyang, Meihu, Shinnan, and Kongliau in Chuangwei Township; Wuyuan, Jenchu, Puchern, and Sanchi in Tungshan Township; and Huhsing, Litzer, and Shiawei in Wuchien Township. Because of the abundance of groundwater (<40 m deep) since the 1940s, and although the implementation of a tap water system was begun in the study area in the 1990s, some residents still drink well water. Well water in the Lanyang Basin was found to have an arsenic concentration ranging from undetectable to 3.59 mg/l, with a wide variation in median arsenic concentrations ranging from undetectable to 0.14 mg/l in various villages. The variation in arsenic levels in well water in the Lanyang Basin was much more striking than the arsenic level in artesian well water of the BFD endemic area in southwestern Taiwan (Chen et al., 1962).

2.2. Study subjects

Names and addresses of all adult residents in the study area were extracted from household records kept in local household registration offices where sociodemographic characteristics including gender, birth date, marital status, education, moving of house, and occupation of all members of every household are registered and annually updated. The selection of study subjects from the household registration system was effective and efficient because of the completeness and accuracy of the registration information. In total, 8102 residents including 4056 men and 4046 women who agreed to participate were interviewed at home from October 1991 to September 1994. Four public health nurses who were well trained in interview techniques and the questionnaire details administered a standardized personal interview based on a structured questionnaire. Information obtained from the interview included history of well water consumption, residential history, sociodemographic characteristics, history of cigarette smoking, history of alcohol consumption, physical activities, history of sunlight exposure, and personal and family histories of hypertension, diabetes, cardiovascular disease, heart disease, and cancer. The information on cigarette smoking that was obtained included the age at which the subject began smoking, the average number of cigarettes smoked per day, and the age at which the subject stopped smoking. Information concerning the age at which habitual alcohol consumption began, the average quantity of alcohol consumed per day, and the age at which the subject stopped consuming alcohol was also obtained. Physical activity level at work was evaluated on the basis of the type of job and hours worked per day.

A detailed history of the villages in which the subject resided and water consumption, including water source and duration of consumption, obtained from the questionnaire interview was used to derive cumulative arsenic exposure from drinking well water. In total, 3901 well water samples (one sample from each household) were collected during home interviews, immediately acidified with hydrochloric acid, and stored at -20 °C until subsequent analysis. Hydride generation combined with flame atomic absorption spectrometry was used to determine arsenic concentration in these samples. The arsenic exposure level of each study subject from drinking well water was derived from the arsenic concentration of the household well water. The cumulative arsenic exposure was derived by multiplying the arsenic concentration in well water (in mg/l) by the duration of drinking well water (in years) during consecutive periods of living in different villages, for example, $\Sigma C_i D_i$ where C_i is the arsenic level in well water of the residence where a given study subject lived during period i and D_i the duration of drinking well water during the same period. In other words, this cumulative index equates the level of arsenic in well water with the duration of drinking the water. Both the cumulative arsenic exposure from drinking well water and the average arsenic concentration in drinking water were available only for those subjects who had a complete history of arsenic exposure from drinking well water throughout their lifetime. For a given subject, these two arsenic exposure indices were classified as unknown if the arsenic level in the well water of any residence throughout the subject's lifetime was not available.

2.3. Determination of serum antioxidant micronutrient levels

Serum levels of retinol and β-carotene and α-tocopherol were measured by high-performance liquid chromatography (HPLC) according to the procedures described previously (Miller et al., 1984). Analysis was carried out using reversed-phase HPLC (L-6200A Hitachi, Tokyo, Japan) with a mobile phase of methanol:acetonitrite:-chloroform of 47:48:5 and multiwavelength monitoring. Retinol was detected at 325 nm, β-carotene at 466 nm, and α-tocopherol at 280 nm. Serum samples retrieved from a −70 °C refrigerator were thawed in dim light at room temperature and assayed the same day. Recovery rates for retinol, β-carotene and α-tocopherol ranged 90.0–105.1% at the lowest concentration and 92.7–111.5% at the highest concentration of the standard solution. The precision (coefficient of variation) of retinol, β-carotene and α-tocopherol ranged 5.1–6.9%. We also used α-tocopherol acetate as the internal control to reduce the systematic error of these tests. The precision for α-tocopherol acetate was 3.3%.

2.4. Determination of urinary arsenic species

To achieve a more accurate assessment of arsenic methylation capability, it is necessary to specifically determine only those arsenic species derived from inorganic arsenic and excreted in urine. Frozen urine samples were tested for levels of AsIII, AsV, MMA, and DMA. They were thawed at room temperature, mixed by ultrasonic waves, and filtered through a Sep-Pak C_{18} column. Arsenic species in 200 μl of urine were separated by HPLC (Waters 501, Waters Associates, Milford, MA) with a Phenomenex column (Nucleosil 10sB, Torrance, CA), then, on-line linked to a hydride generator-atomic absorption spectrometer (HG-AAS) to quantify the levels of various species of inorganic arsenic and its metabolites (Norin and Vahter, 1981). Recovery rates for AsIII, DMA, MMA, and AsV ranged 93.8–102.2% with the detection limits of 0.02, 0.06, 0.07, and 0.10 μg/l, respectively. Freeze-dried urine SRM 2670, containing 480 ± 100 μg/l arsenic was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD) and analyzed together with test urine samples to assess the quality control of the method. A standard value of 507 ± 17 μg/l ($n = 4$) was recorded.

2.5. Determination of serum selenium and urinary selenium and arsenic

Frozen serum and urine samples were thawed at room temperature and mixed by ultrasonic waves. Serum was diluted with 0.1% Triton X-100, and urine was diluted with 0.1% nitric acid, and then modified reagent was added. Selenium concentrations were measured by graphite furnace atomic absorption spectrometry (Perkin-Elmer Model 5100). Average recovery rates were 105.71 and 97.67% and coefficients of variance were 6.43 and 6.05% with detection limits of 0.84 and 0.66 μg/l for selenium and arsenic, respectively. Freeze-dried SRM 1598, containing 42.4 ± 3.5 μg/l selenium was obtained from NIST and analyzed together with test urine samples to assess the quality control of the method. A standard value of 38.8 ± 2.48 μg/l ($n = 5$) was recorded. Standard reference material (Serenorm Teu 009024) containing 100 μg/l arsenic was analyzed together with test urine samples to assess the quality control of the method. A standard value of 103.2 ± 6.24 μg/l ($n = 5$) was recorded.

In addition to the levels of inorganic and organic arsenic in urine, the percentages of AsIII, AsV, MMA, and DMA of total arsenic were also analyzed to examine the arsenic methylation capability of study subjects. An increased percentage of AsIII + AsV of total arsenic and/or a decreased percentage of DMA of total arsenic reflect a decreased methylation capability. The mean and standard error (SE) of urinary levels and percentages of various arsenic metabolites were calculated and analyzed by Student's t-test for different genders, and by ANOVA for differences in age and duration of exposure. As age, gender, and cumulative exposure to arsenic were all related to urinary levels or percentages of various arsenic metabolites, it was essential to examine the correlation with arsenic levels and percentages for a given independent variable when other factors

were adjusted through multivariate analysis. Multiple regression analysis was thus used to evaluate the associations of urinary levels or percentages of various arsenic metabolites with age, gender, serum selenium, urinary arsenic, and selenium levels, and previous cumulative exposure to arsenic through consumption of artesian well water. Net correlation coefficients were also calculated for urinary levels or percentages of various arsenic metabolites and previous cumulative exposure to arsenic.

3. Results

The sociodemographic characteristics of the adult residents who did not participate in physical examinations were similar to those of the participants. As shown in Table 1, more than half of the participants were less than 60 years old. Most study subjects were married, were Fukienese, and had an educational level of elementary school or above. Of study subjects, 35.6% smoked cigarettes and 14.2% consumed alcohol.

Table 1
Demographic characteristics of residents of the Lanyang Basin, Taiwan

Variable	Group	Number	Percent
Gender	Male	113	44.8
	Female	139	55.2
Age (year)	40–49	60	23.8
	50–59	108	42.9
	60–69	76	30.2
	>70	8	3.2
Cigarette smoking	No	165	65.5
	Yes[a]	87	34.5
Alcohol consumption	No	221	87.7
	Yes[b]	31	12.3
Marital status	Married	234	92.9
	Single/divorced/widowed	18	7.1
Ethnicity	Fukienese	247	98.0
	Non-Fukienese	5	2.0
Education level	Illiterate	103	40.9
	Elementary school and above	149	59.1

[a] Cigarette smoking more than 3 days per week.
[b] Alcohol consumption more than 1 day per week.

The percentage of various arsenic metabolites in urine, total As_i, overall As_{i+o}, and serum and urinary selenium by gender, age, cigarette smoking, and alcohol consumption are presented in Table 2. Total As_i, overall As_{i+o}, and urinary selenium were significantly higher in men than in women. Percentages of arsenic metabolites in urine significantly differed between men and women, with higher percentages of MMA in men, but a higher percentage of DMA in women. There were no significant differences among the four age groups in the percentage of various arsenic metabolites in urine, but serum selenium in subjects less than 51 years old was higher than that in the 58–64 age group. Cigarette smokers had higher urinary selenium than nonsmokers, and alcohol consumers had higher inorganic arsenic and MMA percentage and lower DMA percentage than nondrinkers.

Table 3 illustrates univariate regression analysis for inorganic arsenic, MMA, DMA percent, total As_i, and overall As_{i+o}. Overall, As_{i+o} increased significantly with cumulative arsenic exposure, arsenic concentration of well water, α-tocopherol, and urinary selenium. Total As_i was significantly related to cumulative arsenic exposure, arsenic concentration of well water, duration of drinking well water, and α-tocopherol. DMA percent was markedly related to urinary selenium, but inversely related to alcohol consumption. There was no significant relation among MMA percent and other variables. Inorganic arsenic percent showed a negative trend with urinary selenium, but it increased with alcohol consumption.

The data in Table 4 show no statistically significant differences in MMA percent among tertiles of urinary selenium. Inorganic arsenic percent decreased with urinary selenium, but DMA percent, total As_i, and overall As_{i+o} increased with urinary selenium. Inorganic arsenic percent in the 81.3–95.2 μg/l serum selenium group was significantly less than that in the more than 95.2 μg/l serum selenium group. MMA percent, DMA percent, total As_i, and overall As_{i+o} did not significantly differ among tertiles

Table 2
Percent of various arsenic metabolites and total inorganic arsenic metabolites in urine, overall inorganic and organic arsenic in urine, serum and urinary selenium by gender, age, cigarette smoking, and alcohol consumption among healthy residents of the Lanyang Basin, Taiwan

Variable	Group	Number	Inorganic arsenic percent (mean ± SE)	MMA percent (mean ± SE)	DMA percent (mean ± SE)	Total As_i (μg/l) (mean ± SE)	Overall As_{i+o} (μg/l) (mean ± SE)	Serum selenium (μg/l) (mean ± SE)	Urinary selenium (μg/l) (mean ± SE)
Age (year)	≤ 51	65	13.1 ± 1.5	13.5 ± 1.4	73.5 ± 2.3	81.6 ± 11.4	198.4 ± 38.4	101.2 ± 3.4[a]	23.5 ± 2.1
	52–57	69	9.3 ± 1.1	16.8 ± 1.5	74.3 ± 1.9	120.9 ± 18.2	165.5 ± 15.8	91.1 ± 2.3	24.0 ± 1.6
	58–64	71	11.1 ± 1.4	15.3 ± 1.1	74.0 ± 1.8	99.2 ± 13.4	207.0 ± 31.6	86.5 ± 1.8	21.9 ± 1.8
	> 64	48	11.4 ± 1.0	13.6 ± 1.1	75.0 ± 1.5	79.0 ± 11.9	129.3 ± 12.1	91.9 ± 3.4	19.4 ± 1.2
Gender	Male	113	11.8 ± 0.9	16.5 ± 1.1*	72.2 ± 1.5^{+}	123.5 ± 13.4**	218.7 ± 26.9*	94.0 ± 2.3	26.5 ± 1.4**
	Female	139	10.7 ± 0.9	13.6 ± 0.8	75.7 ± 1.3	74.8 ± 6.9	145.9 ± 12.8	91.3 ± 1.7	19.0 ± 1.0
Cigarette smoking	No	165	10.9 ± 0.9	14.2 ± 0.8	75.2 ± 1.2	87.0 ± 8.1^{+}	154.6 ± 11.8^{+}	92.0 ± 1.5	20.1 ± 1.1**
	Yes	87	11.7 ± 0.9	16.1 ± 1.2	72.2 ± 1.6	115.0 ± 14.3	225.4 ± 34.3	93.5 ± 2.8	26.8 ± 1.6
Alcohol consumption	No	221	10.7 ± 0.7*	14.3 ± 0.7*	75.3 ± 1.0**	93.7 ± 7.6	176.8 ± 15.9	92.6 ± 1.5	22.3 ± 1.0
	Yes	31	15.0 ± 2.1	18.8 ± 2.3	66.1 ± 3.3	120.0 ± 23.4	193.3 ± 27.0	92.0 ± 4.0	22.8 ± 2.3
Total subjects		252	11.2 ± 0.6	14.9 ± 0.7	74.2 ± 1.0	96.9 ± 7.3	178.8 ± 14.2	92.5 ± 1.4	22.4 ± 0.9

Total $As_i = As^{3+} + As^{5+} + MMA + DMA$; inorganic arsenic percent = $((As^{3+} + As^{5+})/\text{total } As_i) \times 100$; MMA percent = $(MMA/\text{total } As_i) \times 100$; DMA percent = $(DMA/\text{total } As_i) \times 100$; overall As_{i+o} = overall inorganic and organic arsenic in urine.

[a] The < 51 age group vs. the 58–64 age group by ANOVA and Scheffe's test ($P < 0.001$).

$^{+}$ $0.05 < P < 0.1$.

* $P < 0.05$.

** $P < 0.01$.

Table 3
Univariate analysis of inorganic arsenic, MMA, DMA percent, total inorganic arsenic metabolites in urine, and overall inorganic and organic arsenic in urine among healthy residents of the Lanyang Basin, Taiwan

Variable	Inorganic arsenic percent (β^a) (SE)	MMA percent (β^a) (SE)	DMA percent (β^a) (SE)	Total As_i (β^a) (SE)	Overall As_{i+o} (β^a) (SE)
Cumulative arsenic exposure ((mg/l) × year)	0.08 (0.09)	−0.03 (0.09)	−0.04 (0.13)	4.79 (0.92)**	3.62 (1.90)$^+$
Arsenic concentration of well water (μg/l)	0.005 (0.004)	−0.002 (0.004)	−0.003 (0.006)	0.18 (0.04)**	0.17 (0.08)*
Duration of drinking well water (year)	−0.04 (0.05)	0.03 (0.05)	0.01 (0.08)	1.13 (0.55)*	0.69 (1.09)
Retinol (μg/dl)	0.01 (0.02)	−0.002 (0.02)	−0.01 (0.03)	0.31 (0.24)	0.23 (0.48)
α-Tocopherol (μg/dl)	0.0003 (0.002)	0.0006 (0.002)	−0.0007 (0.003)	0.034 (0.02)$^+$	0.10 (0.04)**
β-Carotene (μg/dl)	−0.04 (0.02)	0.02 (0.02)	0.02 (0.04)	−0.02 (0.27)	0.37 (0.53)
Lycopene (μg/dl)	−0.19 (0.12)	0.09 (0.13)	0.09 (0.19)	−1.22 (1.37)	2.60 (2.71)
Urinary selenium (μg/l)	−0.14 (0.05)**	−0.07 (0.05)	0.21 (0.07)**	0.61 (0.53)	3.44 (1.01)**
Serum selenium (μg/l)	0.03 (0.03)	−0.007 (0.03)	−0.03 (0.04)	0.15 (0.33)	0.34(0.65)
Cigarette smoking (yes vs. no)	0.19 (2.02)	−0.75 (2.05)	−0.99 (2.99)	−19.70 (22.35)	39.82 (43.27)
Alcohol consumption (yes vs. no)	4.87 (2.15)*	3.31 (2.20)	−8.81 (3.23)**	−2.89 (24.10)	26.22 (47.43)

Total $As_i = As^{3+} + As^{5+} + MMA + DMA$; inorganic arsenic percent = $((As^{3+} + As^{5+})/\text{total } As_i) \times 100$; MMA percent = (MMA/total As_i) × 100; DMA percent = (DMA/total As_i) × 100; Overall As_{i+o} = overall inorganic and organic arsenic in urine.

[a] Age-gender-adjusted regression coefficient.

$^+$ $0.05 < P < 0.1$.

* $P < 0.05$.

** $P < 0.01$.

of serum selenium. Inorganic arsenic percent and total As_i in the higher than 4.51 mg/l × year cumulative arsenic exposure group were higher than those of the 1.98–4.51 mg/l × year group, and total As_i in the same group was also higher than that in the less than 1.97 mg/l × year cumulative arsenic exposure group. Inorganic arsenic percent in the higher than 122.58 μg/l arsenic concentration of well water group was higher than those of the 65.23–122.58 μg/l and the less than 65.22 μg/l groups. MMA percent, DMA percent, total As_i, and overall As_{i+o} did not significantly differ among tertiles of arsenic concentration of well water. Inorganic arsenic percent, MMA percent, DMA percent, and overall As_{i+o} did not significantly differ among tertiles of duration of drinking well water, but total As_i in duration of drinking well water for less than 36 years was higher than that in the more than 50 years group. Inorganic arsenic percent in the 580.20–860.21 μg/dl α-tocopherol group was less than that in the more than 860.21 μg/dl group, but DMA percent in the same group was higher than that in the more than 860.21 μg/dl α-tocopherol group. Total As_i in the 580.20–860.21 μg/dl α-tocopherol group was higher than that in the less than 580.19 μg/dl group.

After taking into account other potential confounders, greater cumulative arsenic exposure is associated with results from a higher inorganic arsenic percent, and higher α-tocopherol and urinary selenium is associated with results from a lower inorganic arsenic percent. MMA percent is still unrelated to other variables. Higher urinary selenium is associated with a result of higher DMA percent. DMA percent significantly decreased with alcohol consumption. Total As_i and overall As_{i+o} significantly increased with cumulative arsenic exposure, α-tocopherol, and urinary selenium (Table 5).

Table 6 depicts multivariate analysis for inorganic arsenic, MMA, DMA percent, total As_i, and overall As_{i+o}. The same results were obtained when arsenic concentration of well water replaced

Table 4
Percent of various arsenic metabolites, total inorganic arsenic metabolites, and overall inorganic and organic arsenic in urine by urinary and serum selenium among healthy residents of the Lanyang Basin, Taiwan

Variable	Group	Number	Inorganic arsenic percent (mean ± SE)	MMA percent (mean ± SE)	DMA percent (mean ± SE)	Total As_i (μg/l) (mean ± SE)	Overall As_{i+o} (μg/l) (mean ± SE)
Urinary selenium (μg/l)	≤ 15.2	83	13.48 ± 1.43[a,*]	15.93 ± 1.16	70.93 ± 2.00[a,*]	69.36 ± 9.71[a,*]	115.13 ± 11.03[a,*]
	15.3–25.2	84	10.31 ± 0.97[b,*]	14.10 ± 0.98	75.59 ± 1.45[b,*]	103.66 ± 13.73	157.92 ± 14.19
	> 25.2	85	9.75 ± 0.84	14.64 ± 1.25	75.89 ± 1.51	116.38 ± 13.37	261.52 ± 36.48[c,*]
Serum selenium (μg/l)	≤ 81.2	83	10.70 ± 1.17	15.04 ± 1.01	74.25 ± 1.54	89.06 ± 11.30	177.45 ± 27.12
	81.3–95.2	84	9.72 ± 0.94[d,*]	15.23 ± 1.05	75.34 ± 1.46	86.73 ± 9.98	168.68 ± 19.46
	> 95.2	85	13.09 ± 1.20	14.39 ± 1.33	72.84 ± 2.00	114.20 ± 15.67	190.50 ± 26.85
Cumulative arsenic exposure ((mg/l) × year)	≤ 1.97	83	10.70 ± 1.09	14.39 ± 1.17	75.22 ± 1.61	78.03 ± 9.47	153.35 ± 25.90
	1.98–4.51	84	9.32 ± 0.82	15.38 ± 1.15	75.30 ± 1.51	79.00 ± 8.35	174.40 ± 29.26
	> 4.51	85	13.50 ± 1.33[e,*]	14.89 ± 1.10	71.94 ± 1.90	132.92 ± 17.18[e,f,*]	208.49 ± 16.83
Arsenic concentration of well water (μg/l)	≤ 65.22	83	10.08 ± 0.81	14.92 ± 1.17	75.30 ± 1.25	78.01 ± 9.43	164.70 ± 34.65
	65.23–122.58	84	8.99 ± 1.02	15.91 ± 1.26	75.11 ± 1.80	105.27 ± 12.90	172.11 ± 13.74
	> 122.58	85	14.51 ± 1.36[g,h,*]	13.80 ± 0.95	72.02 ± 1.91	106.38 ± 14.68	199.90 ± 21.20
Duration of drinking well water (year)	≤ 36	90	10.94 ± 1.13	13.91 ± 1.09	75.15 ± 1.64	76.37 ± 8.82[i,*]	154.60 ± 23.71
	37–50	81	12.26 ± 1.32	15.09 ± 1.32	72.98 ± 2.07	99.67 ± 13.38	208.22 ± 31.31
	> 50	81	10.32 ± 0.82	15.77 ± 0.99	74.22 ± 1.24	116.17 ± 15.11	176.09 ± 16.48
α-Tocopherol (μg/dl)	≤ 580.19	84	12.01 ± 1.25	14.91 ± 0.92	73.08 ± 1.67	73.33 ± 8.36	151.61 ± 17.07
	580.20–860.21	84	9.10 ± 0.96[j,*]	13.74 ± 1.16	77.43 ± 1.58[j,*]	114.45 ± 14.57[k,*]	193.88 ± 17.26
	> 860.21	84	12.38 ± 1.09	16.01 ± 1.29	71.94 ± 1.75	102.18 ± 13.60	190.73 ± 34.88

[a] Significantly different from the > 25.2 μg/l urinary selenium group.
[b] Significantly different from the ≦ 15.2 μg/l urinary selenium group.
[c] Significantly different from the 15.3–25.2 μg/l urinary selenium group.
[d] Significantly different from the > 95.2 μg/l serum selenium group.
[e] Significantly different from the 1.98–4.51 (mg/l) × year cumulative arsenic exposure group.
[f] Significantly different from the ≦ 1.97 (mg/l) × year cumulative arsenic exposure group.
[g] Significantly different from the ≦ 65.22 μg/l arsenic concentration of well water group.
[h] Significantly different from the 65.23–122.58 μg/l arsenic concentration of well water group.
[i] Significantly different from the > 50-year duration of drinking well water group.
[j] Significantly different from the > 860.21 μg/dl α-tocopherol group.
[k] Significantly different from α-tocopherol ≦ 580.19 μg/dl α-tocopherol group.
* $P < 0.05$.

Table 5
Multivariate analysis of inorganic arsenic, MMA, DMA percent, total inorganic arsenic metabolites in urine, and overall inorganic and organic arsenic in urine among healthy residents of the Lanyang Basin, Taiwan

Variable	Group	Inorganic arsenic percent (β^a) (SE)	MMA percent (β^a) (SE)	DMA percent (β^a) (SE)	Total As_i (β^a) (SE)	Overall As_{i+o} (β^a) (SE)
Cumulative arsenic exposure (mg/l) × year)	1.98–4.51 vs. ≤1.97	−0.92 (1.78)	2.86 (1.87)	−2.03 (2.70)	36.54 (19.57)$^+$	73.60 (39.24)*
	>4.51 vs. ≤1.97	3.17 (1.72)$^+$	1.44 (1.81)	−4.51 (2.60)$^+$	80.66 (18.87)**	102.34 (37.74)**
	Trend test (*P*-value)	0.06	0.64	0.14	0.0001	0.006
α-Tocopherol (μg/dl)	580.2–860.2 vs. ≤580.2	−3.31 (1.59)*	−0.71 (1.67)	4.33 (2.41)$^+$	57.55 (17.46)**	62.11 (34.94)$^+$
	>860.2 vs. ≤580.2	0.63 (1.81)	2.44 (1.91)	−2.73 (2.75)	62.76 (19.90)**	74.87 (39.87)$^+$
	Trend test (*P*-value)	0.33	0.40	0.29	0.001	0.06
Urinary selenium (μg/dl)	15.3–25.2 vs. ≤15.2	−3.09 (1.54)*	−1.83 (1.63)	4.63 (2.34)*	39.34 (16.97)*	43.20 (33.97)
	>25.2 vs. ≤15.2	−4.45 (1.60)**	−2.54 (1.69)	6.81 (2.42)**	40.45 (17.62)*	135.79 (35.16)**
	Trend test (*P*-value)	0.01	0.16	0.01	0.03	0.0002
Alcohol consumption	Yes vs. no	4.26 (2.12)*	2.95 (2.23)	−7.70 (3.21)*	4.80 (23.30)	−27.34 (46.66)

Total $As_i = As^{3+} + As^{5+} + MMA + DMA$; inorganic arsenic percent $= ((As^{3+} + As^{5+})/\text{total } As_i) \times 100$; MMA percent $= (MMA/\text{ total } As_i) \times 100$; DMA percent $= (DMA/\text{total } As_i) \times 100$; overall As_{i+o} = overall inorganic and organic arsenic in urine.

[a] Each model was adjusted for age, gender, and cigarette smoking.

$^+$ $0.05 < P < 0.1$.

* $P < 0.05$.

** $P < 0.01$.

Table 6
Multivariate analysis of inorganic arsenic, MMA, DMA percent, total inorganic arsenic metabolites in urine, and overall inorganic and organic arsenic in urine among healthy residents of the Lanyang Basin, Taiwan

Variable	Group	Inorganic arsenic percent (β^a) (SE)	MMA percent (β^a) (SE)	DMA percent (β^a) (SE)	Total As_i (β^a) (SE)	Overall As_{i+o} (β^a) (SE)
Arsenic concentration of well water (μg/l)	65.23–122.58 vs. ≤65.22	−0.30 (1.76)	2.20 (1.87)	−2.08 (2.70)	63.45 (19.82)*	68.99 (39.36)**
	>122.58 vs. ≤ 65.22	5.04 (1.73)*	−0.32 (1.84)	−4.59 (2.66)**	63.93 (19.52)*	93.94 (38.68)***
	Trend test (*P*-value)	0.002	0.60	0.12	0.0002	0.02
α-Tocopherol (μg/dl)	580.2–860.2 vs. ≤580.2	−2.86 (1.57)**	−0.98 (1.68)	4.15 (2.42)**	61.44 (17.76)*	63.83 (35.22)**
	>860.2 vs. ≤ 580.2	1.49 (1.79)	1.68 (1.91)	−2.85 (2.50)	68.13 (20.18)*	71.87 (40.06)**
	Trend test (*P*-value)	0.12	0.68	0.26	0.006	0.08
Urinary selenium (μg/l)	5.3–25.2 vs. ≤ 15.2	−2.70 (1.53)**	−2.16 (1.63)	4.60 (2.35)**	35.15 (17.26)***	40.43 (34.25)
	>25.2 vs. ≤15.2	−4.49 (1.58)*	−2.49 (1.69)	6.79 (2.42)*	40.80 (17.86)***	137.50 (35.31)*
	Trend test (*P*-value)	0.01	0.14	0.01	0.04	0.0002
Alcohol consumption	Yes vs. no	3.92 (2.09)**	3.08 (2.23)	−7.50 (3.22)***	1.68 (23.61)	−30.39 (46.86)

Total $As_i = As^{3+} + As^{5+} + MMA + DMA$; inorganic arsenic percent = $((As^{3+} + As^{5+})/\text{total } As_i) \times 100$; MMA percent = $(MMA/\text{total } As_i) \times 100$; DMA percent = $(DMA/\text{total } As_i) \times 100$; overall As_{i+o} = overall inorganic and organic arsenic in urine.

[a] Each model was adjusted for age, gender, and cigarette smoking.

* $P < 0.01$.

** $0.05 < P < 0.1$.

*** $P < 0.05$.

cumulative arsenic exposure in the multiple regression model.

4. Discussion

The level of exposure to inorganic arsenic is often determined by the concentration of its metabolites in urine (ATSDR, 1993). Urinary levels of arsenic species metabolically related to inorganic arsenic are more reliable biomarkers of exposure to inorganic arsenic than is total urinary arsenic, which also contains high levels of organoarsenic compounds derived from dietary intake of seafood (Buchet et al., 1980). In this study, the inorganic arsenic percentage, MMA percentage, and DMA percentage were 11.2 ± 0.6, 14.9 ± 0.7, and 74.2 ± 1.0, respectively. The MMA percent of residents of the Lanyang Basin was lower than that for skin cancer patients ($30.7 \pm 4.59\%$) and healthy controls ($23.00 \pm 1.00\%$) in a BFD endemic area of southwestern Taiwan (Hsueh et al., 1997). The MMA percent was the same as that for residents of San Pedro Town, Chile ($15.0 \pm 5.5\%$) where arsenic concentration of river water was higher than 170 μg As/l, but it was higher than that of residents of Toconao ($10.6 \pm 4.2\%$) where arsenic concentration of river water was 15 μg As/l (Hopenhayn-Rich et al., 1996). We found that women had a significantly higher DMA percent and lower MMA percent than men. Data suggest that women possess a more efficient methylation capability than men as well as do residents of a BFD endemic area in southwestern Taiwan (Hsueh et al., 1998). Unlike the residents of the BFD endemic area where the age effect on arsenic species in urine may reflect a poor methylation ability among the elderly (Hsueh et al., 1998), age was not related to the capability to metabolize inorganic arsenic in this study. It was also observed that a higher arsenic concentration in well water produced higher levels of total As_i and overall As_{i+o}. Long-term ingested inorganic arsenic appears to be deposited in human. This means that cumulative previous exposure to inorganic arsenic through consuming well water, therefore, elevates total arsenic metabolites in urine, which is also supported by an increase in the cumulative previous arsenic exposure.

The impact of dietary selenium status on arsenic metabolism is of interest from the standpoint of both exposure potential and toxicity. Selenium is an essential element both widely and unevenly distributed in the earth's crust. Due to this uneven distribution, crops and forage in some areas of the world provide diets that are either deficient or excessive in selenium for livestock or human. Acute and chronic toxicity associated with excess dietary selenium has been observed in both animals and human in the form of neurological and neuromuscular symptoms and skin lesions. Keshan's disease, a form of cardiomyopathy endemic in certain areas of China, is an example of a syndrome associated with selenium deficiency in human (ATSDR, 1996). Deficient levels of daily intake of selenium which produced Keshan's disease and Kaschin-Beck disease were estimated to be 7–11 μg of selenium per day (Yang, 1987).

An interaction between arsenic and selenium seems to attenuate the toxicity of both metals. Sodium selenite given in combination with sodium arsenite had earlier been observed to decrease chromosome and chromatid breaks induced by the latter in human lymphocyte cultures (Swiens, 1983) and to reduce micronuclei in preimplantation mouse embryos in vivo (Shen et al., 1992). Sodium selenite and selenomethionine were reported to protect against the genotoxic effects induced by arsenic in human peripheral lymphocytes (Hu et al., 1996). The frequency of sister chromatid exchange induced by arsenic was significantly reduced by preincubation with sodium selenite (Hu et al., 1996). Dietary supplementation with sodium selenite 1 h before exposure to sodium arsenite reduced the clastogenic effect of the latter in mice in vivo to a statistically significant level (Biswas et al., 1999). Selenite and trimethylselenonium iodide prevented tetraploidy induced in Chinese hamster ovary cells by exposure to relatively higher concentrations of a major metabolite of inorganic arsenic, DMA (Ueda et al., 1997). Selenite antagonized the induction of heme oxygenase (a stress protein induced by a variety of chemical and physical agents) by arsenite in HeLa cells (Taketani et al., 1991).

Selenite suppressed the teratogenic effects of inorganic arsenic in hamsters (Holmberg and Ferm, 1960). Both arsenic and selenium behave as metabolic antipodes, i.e. each can be used to alleviate the symptoms of poisoning of the other. They directly interact with each other and compete for methyl groups (Schrauzer, 1992).

Levander and Baumann (1966) reported that treatment with selenite altered the in vivo retention and distribution of inorganic arsenic. Gregus et al. (1998) hypothesized that formation of cholephilic ternary complexes of GSH with selenol metabolites of selenite and with arsenic is responsible for the increased biliary excretion of selenium and arsenic in animals concurrently exposed to these metalloids. Such a complex, seleno-bis(*S*-glutathionyl)arsinium ion, has recently been identified in the bile of rabbits injected with selenite and arsenite (Gailer et al., 2000). Mice consuming a selenium-supplemented diet excreted a significantly higher percentage of an oral dose of arsenate in the urine with a lower ratio of methylated arsenicals to inorganic arsenic than did mice that consumed a selenium-adequate diet (Kenyon et al., 1997). This suggests that inorganic arsenic methylation is suppressed by high selenium intake. Selenite has also been reported to inhibit methylation of inorganic arsenic in an in vitro system that contained rat liver cytosol (Buchet and Lauwerys, 1985). Selenite was also a potent inhibitor of arsenic methyltransferase purified from rabbit liver (Zakharyan et al., 1995). Coexposure to selenite results in significant accumulation of inorganic arsenic (and in some cases also MMA) in cultured cells (Styblo and Thomas, 2001). The presence of decreased DMA/MMA ratios in rat hepatocytes coexposed to selenite suggests that the conversion of MMA to DMA is preferentially inhibited by selenite. The first methylation reaction, the conversion of inorganic arsenic to MMA, is less sensitive to inhibition by selenite, while the second methylation reaction is more sensitive than the first methylation reaction to the inhibitory effects of selenite (Styblo et al., 1996, 2000; Styblo et al., 2001). Concurrent exposure to selenite interferes with the metabolism of arsenite and exacerbates the cytotoxic effects of arsenite and its trivalent methylated metabolites (methylarsonous acid and dimethylarsinous acid) in cultured cells. The increased cytotoxicity of these species may be linked to increased retention in cells. Dietary supplementation or therapeutic treatment with selenium is currently being considered as a prophylactic or therapeutic measure for populations chronically exposed to high levels of inorganic arsenic in drinking water and food.

Blood selenium levels vary widely in different localities depending on the intake of selenium (Iyengar and Woittiez, 1988), but the blood levels are usually <0.2 mg/l (Xu et al., 1994). For the activity of cytosolic glutathione peroxidase, an indicator of selenium repletion, the estimated optimum concentration of selenium in the serum is 0.1 mg selenium/l (Thomson et al., 1993). In population with a normal intake of selenium and without exposure to selenium, the usual urine selenium is <0.03 mg selenium/l (Robberrecht and Deelsta, 1984). Concentrations of selenium in the hair, serum, and urine were shown to reflect the selenium supplied to the body, and they decreased to the lowest level when a selenium-deficient diet was consumed, while they increased rapidly when the concentration of dietary selenium in any form was increased (Shiobara et al., 1998). It was also indicated that selenium absorbed in either chemical form was excreted mainly into the urine, depending on the selenium dose. As a result, the urinary amount of selenium appears to reflect the total amount of selenium absorbed from the diet (Shiobara et al., 1998). Our data delineated the average level of serum and urinary selenium to be approximately 92 and 22 μg/l, respectively, which suggests that dietary selenium intake is not low in this population. Serum selenium levels were significantly correlated with serum nutritional parameters (total cholesterol, triglyceride, retinol, and α-tocopherol). In the multivariate analysis, these confounding factors were adjusted, and our data still show that the urinary arsenic level was significantly correlated with arsenic concentration of well water and with urinary selenium. After adjusting for other risk factors, serum α-tocopherol was significantly associated with urinary arsenic level and total arsenic. One possible explanation is that better nutritional status can enhance arsenic elimination. In this study, we

demonstrate that α-tocopherol is significantly related to serum selenium. In vitro vitamin E protects cell membranes from oxidative degradation due to a selenium deficiency, presumably by its own antioxidant activity (Combs and Scott, 1977; Diplock, 1978). Strong adverse effects of low selenium among individuals who also have low vitamin E levels are expected (Willett et al., 1983). Selenium is a component of glutathione peroxidase, and this enzyme may be important for vitamin E function (Hoeksstra, 1975). We also observed that the inorganic arsenic percent was inversely associated with the urinary selenium level, but DMA percent appeared to have a significant positive association with urinary selenium level. This finding suggests that urinary selenium may increase the elimination of arsenic and alter the profile of urinary arsenic metabolites. One study showed that arsenic and selenium are concentrated and precipitated in lysosomes of renal cells in the form of insoluble selenide (As_2Se) in rats, and that their precipitates are excreted in the urine (Berry and Galle, 1994). Sodium selenite has been suggested to mobilize arsenic from tissues and increase its excretion from arsenite- and arsenate-poisoned rats (Himley et al., 1991). Another experimental study provided suggestive evidence that a diet deficient or excessive in selenium may alter arsenate disposition and methylation (Kenyon and Hughes, 1997). It is possible that arsenic elimination is delayed in a selenium-deficient status because the supply of glutathione is limited as it reduces arsenate to arsenite and is also involved in analogous reduction reactions with pentavalent MMA and DMA (Scott et al., 1993; Thompson, 1993; Delnomdedieu et al., 1994). The selenium concentration in the urine showed an inverse pattern to the arsenic concentration in blood and hair in BFD patients (Wang et al., 1993, 1994; Wang, 1996). This may explain why a lower dietary intake of selenium causes retention of arsenic in the body.

In summary, these studies provide suggestive evidence that higher urinary selenium levels reflecting a greater dietary intake of selenium may alter arsenic disposition and methylation. Further studies focusing on the mechanism of arsenic methylation are needed to confirm these findings.

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