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Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in blackfoot disease-hyperendemic villages in Taiwan

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Abstract

Long-term exposure to ingested inorganic arsenic is associated with peripheral vascular disease (PVD) in the blackfoot disease (BFD)-hyperendemic area in Taiwan. This study further examined the interaction between arsenic exposure and urinary arsenic speciation on the risk of PVD. A total of 479 (220 men and 259 women) adults residing in the BFD-hyperendemic area were studied. Doppler ultrasound was used to diagnose PVD. Arsenic exposure was estimated by an index of cumulative arsenic exposure (CAE). Urinary levels of total arsenic, inorganic arsenite (As^{III}) and arsenate (As^{V}), monomethylarsonic acid (MMA^{V}), and dimethylarsinic acid (DMA^{V}) were determined. Primary methylation index [PMI = $MMA^{V}/(As^{III} + As^{V})$] and secondary methylation index (SMI = DMA^{V}/MMA^{V}) were calculated. The association between PVD and urinary arsenic parameters was evaluated with consideration of the interaction with CAE and the confounding effects of age, sex, body mass index, total cholesterol, triglycerides, cigarette smoking, and alcohol consumption. Results showed that aging was associated with a diminishing capacity to methylate inorganic arsenic and women possessed a more efficient arsenic methylation capacity than men did. PVD risk increased with a higher CAE and a lower capacity to methylate arsenic to DMA^{V}. The multivariate-adjusted odds ratios for CAE of 0, 0.1–15.4, and >15.4 mg/L × year were 1.00, 3.41 (0.74–15.78), and 4.62 (0.96–22.21), respectively ($P < 0.05$, trend test); and for PMI ≤ 1.77 and SMI > 6.93, PMI > 1.77 and SMI > 6.93, PMI > 1.77 and SMI ≤ 6.93, and PMI ≤ 1.77 and SMI ≤ 6.93 were 1.00, 2.93 (0.90–9.52), 2.85 (1.05–7.73), and 3.60 (1.12–11.56), respectively ($P < 0.05$, trend test). It was concluded that individuals with a higher arsenic exposure and a lower capacity to methylate inorganic arsenic to DMA^{V} have a higher risk of developing PVD in the BFD-hyperendemic area in Taiwan.

Keywords: Arsenic exposure; Urinary arsenic speciation; Peripheral vascular disease

Introduction

About 40 million people in different parts of the world are exposed to arsenic via drinking water (Nordstrom, 2002). Exposure to inorganic arsenic through drinking artesian water is the most possible cause of the so-called blackfoot disease (BFD), a unique peripheral vascular disease (PVD) identified in the endemic area along the southwestern coast of Taiwan (Tseng, 1989). The disease frequently ends up with dry gangrene and spontaneous amputation of affected extremities, with an underlying pathological change of severe systemic atherosclerosis (Tseng, 1989).

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Because atherosclerosis is an insidious process, the circulation of the lower extremity is better assessed by more sophisticated laboratory examinations than its severe clinical manifestations as BFD. Previous studies have shown an abnormal drop of ankle pressures after treadmill exercise, an abnormal decrease in cutaneous microperfusion, and a dose-responsive pattern between ingested inorganic arsenic and PVD in residents of the BFD-hyperendemic area in Taiwan (Tseng, 2002; Tseng et al., 1994, 1995, 1996, 1997). The arsenic-induced PVD was also observed among residents in Chile and Mexico, Moselle vintners, and copper smelter workers (Engel et al., 1994). Residents in the BFD areas also have a significantly increased risk of (Tseng et al., 2003) mortality from (Chen et al., 1996) ischemic heart disease and stroke (Chiou et al., 1997b). Therefore, arsenic-induced atherosclerosis can be consistently demonstrated in epidemiologic studies and the process is systemic. The occurrence of BFD decreased dramatically over the past 2–3 decades after tap water supply was implemented in the endemic region (Tseng, 2002). A recent study also showed that ischemic heart disease mortality declined gradually about 17–21 years after the cessation of consumption of high-arsenic artesian well water in the BFD areas (Chang et al., 2004). These findings strengthen the likelihood that an association exists between arsenic exposure and the development of atherosclerotic diseases. A recent study treating $ApoE^{-/-}$/$LDLr^{-/-}$ mice with 133 μM (10 ppm) sodium arsenite in drinking water for 18 weeks has successfully induced a significant increase in atherosclerotic plaques in the innominate artery compared to controls (Bunderson et al., 2004). This animal model provided evidence for biological plausibility of arsenic-induced atherosclerosis observed in humans in epidemiologic studies.

In drinking water, arsenic is usually found in the form of inorganic arsenate (As^V) or arsenite (As^{III}), depending on the pH and the presence of oxidizing and reducing substances (Andreae, 1977; Shraim et al., 2002). The metabolism of inorganic arsenic involves 2 steps of chemical reactions: reduction and oxidative methylation (Kitchin, 2001; Styblo et al., 2002; Thompson, 1993; Thomas et al., 2001, 2004; Vahter, 2002). Arsenate is reduced to arsenite before it can be further metabolized. Arsenite is then oxidatively methylated to monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V). The methylation process of arsenic is catalyzed by a 42-kDa protein encoded by the *cyt19* genes of mouse and human genomes and the methyl donor has been identified as *S*-adenosylmethionine (Thomas et al., 2004). Previously, methylation of inorganic arsenic has always been considered as a detoxification mechanism because MMA^V and DMA^V have relatively low toxicity (Yamauchi and Fowler, 1994) and are rapidly excreted in the urine (Gebel, 2002; Vahter, 2002). However, recent studies have confirmed the existence of trivalent intermediates and products of monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}), which are more toxic than inorganic arsenite (Kitchin, 2001; Styblo et al., 2002; Thomas et al., 2001). The capacity to metabolize inorganic arsenic differs among individuals; and its biologic effects on various organ systems depend not only on the ingested dosage, but also on the capacity of the individuals to metabolize and detoxify the related compounds. To achieve a more accurate assessment of arsenic methylation capacity, it is necessary to determine the specific arsenic species derived from inorganic arsenic, which are excreted in the urine. Studies evaluating the association between various urinary arsenic metabolites and clinical outcomes are still rare. Some recent studies have documented that subjects with higher cumulative arsenic exposure (CAE) and higher urinary MMA^V percentage or lower urinary DMA^V percentage suffered from a higher risk of skin cancer (Chen et al., 2003a; Hsueh et al., 1997) and bladder cancer (Chen et al., 2003b) among residents of the BFD areas. Our previous studies evaluating the association between inorganic arsenic and PVD have focused on the estimated ingested dosage of total arsenic from drinking water (Tseng et al., 1996, 1997). Whether the metabolism of arsenic could have an effect on the risk of developing PVD is an interesting issue that has not been studied before. Therefore, the present study aimed at evaluating the impact of the interaction between arsenic exposure dosage and urinary arsenic species on the development of PVD among residents in the BFD-hyperendemic area.

Materials and methods

Study subjects. The study subjects were recruited from residents in three BFD-hyperendemic villages in Putai Township of Chiayi County located along the southwest coast of Taiwan. BFD prevalence ranged from 9.6 to 13.6 per 1000 in these villages (Wu et al., 1961), with a median arsenic concentration of artesian well water ranging from 0.70 to 0.93 mg/L (Kuo, 1964). A tap water supply system was implemented in the study villages in the early 1960s, but its coverage remained low until the early 1970s. Artesian well water was no longer used for drinking and cooking after the mid-1970s. The recruitment process of a cohort for follow-up studies on arsenic-induced health hazards was described in details previously (Chen et al., 1995). The population with an age of 30 years or older in the studied villages as registered in the household registration office was 2258. Among them, 1571 (70%) were eligible and lived in the study villages 5 or more days a week. From September to December 1988, a total of 1081 (69%) of the eligible subjects were interviewed. All of the 1081 subjects were invited to participate in the first health examination during January and February 1989 and 941 (87%) subjects actually participated. Bi-annual health examinations were then carried out. The urinary samples used for the assay of arsenic metabolites in the present

study were collected during the first health examination. The Doppler ultrasound examination for diagnosis of PVD was performed and blood samples were collected during the third health examination in February 1993. A total of 479 subjects having both urinary samples and receiving Doppler ultrasound examination were recruited for the present study.

Questionnaire interview and blood sample collection. Two public health nurses who were well trained on interview techniques and well acknowledged of the questionnaire details carried out standardized personal interviews based on a structured questionnaire. Information obtained included history of consuming high-arsenic artesian well water, residential history, and lifestyle variables including alcohol drinking and cigarette smoking. CAE (in mg/L × year) was derived from the arsenic concentration in artesian well water (mg/L) and the duration of consuming the artesian well water (year) as described elsewhere (Chen et al., 1995). Fasting blood samples were also collected for the measurement of serum total cholesterol and triglycerides in the third bi-annual health examination in 1993 (Tseng et al., 1997).

Diagnosis of PVD. During the third health examination in February 1993, 582 subjects including 263 men and 319 women underwent Doppler ultrasound examination (Tseng et al., 1996). The description of such examination was given in detail elsewhere (Tseng et al., 1996, 1997). In brief, systolic pressures on bilateral brachial, posterior tibial, and dorsal pedal arteries were measured with Medacord PVL (Meda-Sonic Inc., Mountain View, CA) and the device automatically calculated the right and left ankle-brachial indices (ABI). Diagnosis of PVD was based on an ABI < 0.90 on either side (Tseng et al., 1996, 1997).

Determination of urinary arsenic species. During the first health examination in 1989, overnight urinary samples were collected from 7:00 PM to the next morning after waking up. Before starting to collect the urinary sample, the subject was instructed to pass urine, which was not collected; and the first void urine after waking up in the next morning was collected. Urinary samples were stored at −20 °C without any additive. The samples were retrieved for the determination of urinary arsenic species within 6 months after collection.

Urine often contains high concentrations of organic arsenic from dietary sources of marine origin such as arsenobetaine and arsenocholine, which are non-toxic, chemically stable, and excreted unchanged (Sakurai et al., 2004). To achieve a more accurate assessment of arsenic methylation capacity, it is necessary to determine specifically only those arsenic species derived from inorganic arsenic and excreted in the urine (Murer et al., 1992). Because the seafood-derived organoarsenic compounds are excreted without metabolic transformation and undetectable by hydride generator-atomic absorption spectrometer (HGAAS), it is generally believed that the HGAAS method may exclude the contribution of all seafood arsenic on urinary arsenic levels.

The frozen urinary samples were thawed at room temperature, dispersed by ultrasonic wave, filtered through Sep-Pak C_{18} column (Mallinckrodt Baker In., NJ, USA), and tested for levels of As^{III}, As^{V}, MMA^{V}, and DMA^{V}. An aliquot of 200 μL was used for separation of arsenic species by HPLC (Waters 501, Waters Associates, Milford, MA, USA) with columns obtained from Phenomenex (Nucleosil 10SB, Torrance, CA, USA). The levels of various species of inorganic arsenic and their metabolites were quantified by HGAAS (Hsueh et al., 1998). Recovery rates for As^{III}, DMA^{V}, MMA^{V}, and As^{V} ranged from 93.8% to 102.2% with detection limits of 0.02, 0.06, 0.07, and 0.10 μg/L, respectively. Freeze-dried urine SRM 2670, which was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and contained 480 ± 100 μg/L of arsenic, was analyzed together with urinary samples of subjects to control for quality. A standard value of 507 ± 17 μg/L (n = 4) was recorded.

Data analyses and statistical methods. Arsenic methylation capacity was assessed by primary methylation index (PMI), defined as the ratio between MMA^{V} and inorganic arsenic (As^{III} + As^{V}) level, and secondary methylation index (SMI), as the ratio between DMA^{V} and MMA^{V}. Student's t test was used to compare differences of urinary arsenic profile between men and women, smokers and non-smokers, alcoholic consumers and non-consumers, and presence or absence of PVD. Multiple linear regression was used to predict the urinary arsenic profile using all potential confounders and using age, sex, BMI, CAE, alcohol drinking, and cholesterol as independent variables in the models. These analyses allowed us to find out the factors associated with the respective urinary arsenic parameters, taking into account the effect of other variables entered simultaneously into the models as independent variables. Logistic regression models were further used to estimate the multivariate-adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) for PVD with regards to CAE and urinary arsenic profile. To clarify the interaction between arsenic exposure and arsenic methylation capacity on PVD, subgroups of CAE vs. urinary total arsenic, CAE vs. inorganic arsenic percentage, CAE vs. MMA^{V} percentage, CAE vs. DMA^{V} percentage, CAE vs. PMI, CAE vs. SMI, and CAE plus PMI vs. SMI were entered as independent variables. The cutoff points for urinary total arsenic, inorganic arsenic percentage, MMA^{V} percentage, DMA^{V} percentage, PMI, and SMI were the respective medians. The logistic regression analyses allowed us to estimate the relative risk (in terms of odds ratio) for a subgroup of subjects with specific exposure dosage of arsenic and urinary arsenic profile relative to the subgroup used as referents denoted with odds ratios of 1.00.

Results

Table 1 compares the urinary arsenic profile between subgroups of sex, cigarette smoking, alcohol drinking, and PVD. A higher urinary total arsenic and MMA^V percentage was observed for men; and a higher DMA^V percentage and SMI for women, indicating a more complete methylation capacity of arsenic to DMA^V in women. Cigarette smokers and alcohol drinkers had higher urinary total arsenic than non-smokers and non-drinkers, respectively. Cigarette smokers also had a significantly higher MMA^V percentage but a lower SMI than non-smokers. None of the urinary arsenic profile differed significantly between subjects with and without PVD.

Table 2 shows the results of the regression analyses. Aging was associated with a lower excreted amount of arsenic and a lower capacity to methylate arsenic as manifested by a lower urinary total arsenic together with a higher MMA^V percentage, a lower DMA^V percentage, and a lower SMI. Women possessed a lower excreted amount of arsenic and a higher capacity to methylate arsenic than men, as manifested by lower urinary total arsenic together with a lower MMA^V percentage, a higher DMA^V percentage, and a higher SMI. CAE was positively predictive for urinary total arsenic (borderline significant), DMA^V, and PMI (borderline significant), and negatively predictive for MMA^V. BMI was positively predictive for DMA^V and negatively predictive for MMA^V. Alcohol consumption and cholesterol were predictive for higher urinary total arsenic, but cholesterol was also predictive for PMI negatively (borderline significant). Additional analyses including cigarette smoking and triglyceride did not show significant prediction for either factor for any of the urinary arsenic profile (data not shown).

Table 3 shows the multivariate-adjusted ORs for PVD. Significant trend tests were observed in most of the 7 models except model 1 (non-significant) and model 4 (borderline significant), indicating increasing risk of PVD with increasing arsenic exposure along with increasing urinary total arsenic (model 1, non-significant trend), increasing inorganic arsenic percentage (model 2), increasing MMA^V percentage (model 3), decreasing DMA^V percentage (model 4, borderline significant trend), increasing PMI (model 5), and decreasing SMI (model 6). However, the lowest risk of PVD was observed in subjects with PMI $\leq$ 1.77 and SMI > 6.93. When this group of subjects was used as the referent group, the risk of PVD increased in subjects having a higher PMI and/or a lower SMI (model 7, Table 3), indicating a more complete secondary methylation of arsenic is associated with the lowest risk. The multivariate-adjusted odds ratios for CAE of 0, 0.1–15.4, and >15.4 mg/L × year were 1.00, 3.41 (0.74–15.78), and 4.62 (0.96–22.21), respectively ($P < 0.05$, trend test); and for PMI $\leq$ 1.77 and SMI > 6.93, PMI > 1.77 and SMI > 6.93, PMI > 1.77 and SMI $\leq$ 6.93, and PMI $\leq$ 1.77 and SMI $\leq$ 6.93 were 1.00, 2.93 (0.90–9.52), 2.85 (1.05–7.73), and 3.60 (1.12–11.56), respectively ($P < 0.05$, trend test).

Discussions

Our previous studies in the BFD-hyperendemic area have disclosed a dose-response relationship between PVD and ingested inorganic arsenic from artesian well water, independent of age, sex, lipid profile, and other traditional risk factors of atherosclerosis (Tseng et al., 1996, 1997).

Table 1
Comparison of urinary arsenic profile between subgroups of sex, cigarette smoking, alcohol drinking, and PVD

Variable	No.	Urinary total arsenic (μg/L)	Inorganic arsenic percentage	MMA^V percentage	DMA^V percentage	PMI	SMI
Sex							
Male	220	88.15 ± 4.21	7.84 ± 0.41	16.33 ± 0.62	75.83 ± 0.80	3.33 ± 0.58	7.37 ± 0.44
Female	259	65.27 ± 2.52	7.94 ± 0.46	11.56 ± 0.46	80.50 ± 0.71	2.51 ± 0.45	12.03 ± 1.12
P		<0.0001	NS	<0.0001	<0.0001	NS	0.0001
Cigarette smoking							
No	366	72.42 ± 2.55	8.01 ± 0.38	13.29 ± 0.43	78.70 ± 0.62	2.62 ± 0.32	10.11 ± 0.79
Yes	103	87.11 ± 6.45	7.68 ± 0.54	15.71 ± 0.93	76.61 ± 1.17	3.83 ± 1.21	7.98 ± 0.70
P		0.0359	NS	0.0114	NS	NS	0.0443
Alcohol drinking							
No	413	72.85 ± 2.35	8.04 ± 0.35	13.74 ± 0.41	78.22 ± 0.58	2.95 ± 0.41	9.61 ± 0.71
Yes	57	96.66 ± 10.64	7.08 ± 0.65	14.56 ± 1.36	78.36 ± 1.72	2.54 ± 0.25	9.72 ± 1.15
P		0.0327	NS	NS	NS	NS	NS
PVD							
No	425	76.45 ± 2.63	8.00 ± 0.34	13.69 ± 0.42	78.31 ± 0.57	2.63 ± 0.31	10.05 ± 0.72
Yes	54	70.46 ± 5.77	7.07 ± 0.67	14.19 ± 1.22	78.74 ± 1.60	4.93 ± 2.10	8.60 ± 0.87
P		NS	NS	NS	NS	NS	NS

Data are mean ± SE.
Inorganic arsenic percentage = ((arsenite + arsenate) / total urinary arsenic) × 100.
DMA^V percentage = (DMA^V / total urinary arsenic) × 100.
MMA^V percentage = (MMA^V / total urinary arsenic) × 100.
PMI = primary methylation index = MMA^V / (arsenite + arsenate).
SMI = secondary methylation index = DMA^V/MMA^V.

Table 2
Multiple linear regression analyses using urinary total arsenic, inorganic arsenic percentage, MMA^V percentage, DMA^V percentage, PMI, and SMI as dependent variables

Dependent/ independent variables	Coefficient	Standard error	*P* value
Urinary total arsenic			
Age (years)	−1.27	0.41	0.0021
Sex (women vs. men)	−19.61	6.40	0.002
CAE (mg/L × year)	0.65	0.38	0.09
BMI (kg/m^2)	−1.14	0.91	0.213
Alcohol consumption (yes vs. no)	20.42	9.29	0.029
Cholesterol (mg/dL)	0.18	0.08	0.025
Inorganic arsenic percentage			
Age (years)	0.01	0.05	0.786
Sex (women vs. men)	0.43	0.78	0.583
CAE (mg/L × year)	−0.07	0.05	0.124
BMI (kg/m^2)	−0.02	0.11	0.848
Alcohol consumption (yes vs. no)	−0.76	1.13	0.50
Cholesterol (mg/dL)	0.02×10^{-2}	0.09×10^{-1}	0.983
MMA^V percentage			
Age (years)	0.29	0.06	0.0001
Sex (women vs. men)	−4.14	0.95	0.0001
CAE (mg/L × year)	−0.13	0.06	0.018
BMI (kg/m^2)	−0.43	0.14	0.002
Alcohol consumption (yes vs. no)	−1.25	1.38	0.364
Cholesterol (mg/dL)	0.01×10^{-1}	0.01	0.880
DMA^V percentage			
Age (years)	−0.30	0.09	0.0006
Sex (women vs. men)	3.72	1.34	0.006
CAE (mg/L × year)	0.20	0.08	0.011
BMI (kg/m^2)	0.45	0.19	0.019
Alcohol consumption (yes vs. no)	2.02	1.95	0.30
Cholesterol (mg/dL)	-0.02×10^{-1}	0.02	0.906
PMI			
Age (years)	−0.02	0.05	0.717
Sex (women vs. men)	−1.33	0.81	0.103
CAE (mg/L × year)	0.09	0.05	0.057
BMI (kg/m^2)	0.01	0.11	0.911
Alcohol consumption (yes vs. no)	−1.32	0.17	0.263
Cholesterol (mg/dL)	−0.02	0.01	0.078
SMI			
Age (years)	−0.29	0.11	0.009
Sex (women vs. men)	4.43	1.72	0.010
CAE (mg/L × year)	0.07	0.10	0.490
BMI (kg/m^2)	−0.11	0.25	0.662
Alcohol consumption (yes vs. no)	2.22	2.50	0.376
Cholesterol (mg/dL)	−0.02	0.02	0.475

Inorganic arsenic percentage = ((arsenite + arsenate) / total arsenic) × 100.
DMA^V percentage = (DMA^V / total arsenic) × 100.
MMA^V percentage = (MMA^V / total arsenic) × 100.
PMI = primary methylation index = MMA^V / (arsenite + arsenate).
SMI = secondary methylation index = DMA^V / MMA^V.
CAE: cumulative arsenic exposure.

Because only a small proportion of the exposed residents would develop PVD, individual capacity to metabolize and detoxify the ingested inorganic arsenic was believed to play an important role on disease development. This individual susceptibility may involve genetic and/or acquired factors.

The proportional levels of urinary arsenic species can be used as indicators for the metabolism of inorganic arsenic. The percentage of inorganic arsenic in urine represents the

Table 3
Multivariate-adjusted odds ratios for PVD with regards to CAE and urinary arsenic profile

Arsenic exposure index	Urinary arsenic profile		Multivariate adjusted odds ratio (95% confidence interval)
Model 1			
CAE (mg/L × year)	Urinary total arsenic (μg / L)		
0	≤64.33 or >64.33		1.00[a]
>0	≤64.33		3.34 (0.60–12.80)
>0	>64.33		3.84 (0.86–17.25)[b]
Model 2			
CAE (mg/L × year)	Inorganic arsenic percentage		
0	≤6.13 or >6.13		1.00[a,*]
>0	≤6.13		2.78 (0.60–12.80)
>0	>6.13		4.62 (1.04–20.84)*
Model 3			
CAE (mg/L × year)	MMA^V percentage		
0	≤11.42 or >11.42		1.00[a,*]
>0	≤11.42		2.64 (0.56–12.45)
>0	>11.42		4.57 (1.01–20.61)*
Model 4			
CAE (mg/L × year)	DMA^V percentage		
0	>81.01 or ≤81.01		1.00[a,b]
>0	>81.01		3.16 (0.69–14.52)
>0	≤81.01		4.12 (0.91–18.69)[b]
Model 5			
CAE (mg/L × year)	PMI		
0	≤1.77 or >1.77		1.00[a,*]
>0	≤1.77		2.91 (0.62–13.59)
>0	>1.77		4.32 (0.96–19.53)[b]
Model 6			
CAE (mg/L × year)	SMI		
0	>6.93 or ≤6.93		1.00[a,*]
>0	>6.93		2.61 (0.56–12.28)
>0	≤6.93		4.61 (1.02–20.76)*
Model 7			
CAE (mg/L × year)			
0			1.00[a,*]
0.1–15.4			3.41 (0.74–15.78)
>15.4			4.62 (0.96–22.21)[b]
	PMI	SMI	
	≤1.77	>6.93	1.00[a,*]
	>1.77	>6.93	2.93 (0.90–9.52)[b]
	≤1.77	≤6.93	2.85 (1.05–7.73)*
	≤1.77	≤6.93	3.60 (1.12–11.56)*

CAE: cumulative arsenic exposure.
Inorganic arsenic percentage = ((arsenite + arsenate) / total arsenic) × 100.
MMA^V percentage = (MMA^V / total arsenic) × 100.
DMA^V percentage = (DMA^V / total arsenic) × 100.
PMI = primary methylation index = MMA^V / (arsenite + arsenate).
SMI = secondary methylation index = DMA^V / MMA^V.
Cutoffs for inorganic arsenic percentage, MMA^V percentage, DMA^V percentage, PMI and SMI are the respective medians.
[a] Trend test.
[b] $0.05 < P < 0.1$.
* $P < 0.05$.

unchanged proportion of the ingested dosage, while the percentages of MMA^V (or PMI) and DMA^V (or SMI) in urine may represent the activity of the first and second methylation phase, respectively. Urine DMA^V percentage has been regarded as an indicator of methylation efficiency (Vahter, 1999a). Arsenic metabolites in the urine of various population groups have been reported to be fairly constant initially, i.e., 10–30% inorganic arsenic, 10–20% MMA^V, and 60–80% DMA^V (Vahter, 1999b). However, ethnical differences have clearly been shown recently. Indigenous people living in the Andes, mainly Atacamenos, excrete only a few percent of MMA^V (2.1% in women to 3.6% in children) (Vahter, 1999b); in the Yaqui Valley, Sonora, Mexico, adults exposed to arsenic-containing water excrete 7.5–9.7% MMA^V in the urine (Meza et al., 2004); but the Taiwanese seem to have an unusually high percentage of MMA^V in urine, with an average of 20–30% (Chiou et al., 1997a; Hsueh et al., 1998). This ethnical difference in the distribution of urinary arsenic species can be further confirmed by a study that simultaneously compared the urinary arsenic species among different ethnicities exposed to comparable dosages of arsenic in high and low arsenic exposure groups in the people of China, Mexico, and Chile (Loffredo et al., 2003). Subjects with lower MMA^V in urine may have faster elimination of ingested arsenic compared to those with higher MMA^V (Vahter, 2002). An inhibition of the second methylation step with higher MMA^V percentage and lower DMA^V percentage in urine has been reported to correlate with increasing arsenic exposure and higher rates of skin lesions in Mexico (Del Razo et al., 1997; Hopenhayn-Rich et al., 1996b). In Taiwan, people showing arsenic-related skin lesions also showed higher inorganic arsenic and MMA^V percentage and lower DMA^V percentage than the matched controls (Yu et al., 2000). Increased MMA^V percentage or decreased DMA^V percentage has also been shown to be associated with a higher risk of skin cancer (Chen et al., 2003a; Hsueh et al., 1997) and bladder cancer (Chen et al., 2003a, 2003b) in Taiwan, even after considering the exposure dosage. Similarly, a Finnish study showed that the number of structural chromosomal aberrations in peripheral lymphocytes was associated with increasing urinary MMA^V but decreasing DMA^V (Maki-Paakkanen et al., 1998). The present study further confirmed the association between PVD and increased MMA^V percentage and/or decreased DMA^V percentage, taking into account the ingested arsenic dosage (Table 3). Therefore, the results of this study suggested that PVD susceptibility is not only related to the exposure dosage of arsenic, the metabolism of arsenic has a significant and great impact on the susceptibility and development of PVD in subjects chronically exposed to arsenic: the more efficient to methylate to DMA^V, the lower the risk.

The importance of a complete second methylation is also implicated from the observation that human beings excrete significant amounts of MMA^V in the urine and are more susceptible to arsenic-related health hazards compared to animals like mice and hamsters that can methylate arsenic very efficiently, resulting in urinary excretion of MMA^V in less than 10% of the urinary arsenic species (Marafante et al., 1987). The more efficient methylation of arsenic has also been suggested as the explanation for the lower sensitivity to arsenic-induced tumor in rodents (WHO, 1980).

Children seem to have more active second methylation capacity than adults, with lower urinary inorganic arsenic and MMA^V, and higher DMA^V along with a higher SMI than adults in a study in Bangladesh (Chowdhury et al., 2003). But similar results were not shown by others (Vahter, 1999a, 1999b). A slight increase of DMA^V with age in adults exposed to arsenic in Finland (Kurttio et al., 1998) but no age or sex differences in children (Buchet et al., 1980; Kalman et al., 1990) was observed in Argentina. On the contrary, MMA^V percentage increased and DMA^V percentage and SMI decreased with age significantly in the present study (Table 2), suggesting that decreasing second methylation capacity is associated with increasing age. This observation was similar to that observed in Bangladesh (Chowdhury et al., 2003). Women had a higher DMA^V percentage and SMI, and a lower urinary total arsenic and MMA^V percentages than men had (Tables 1 and 2), indicating that women possessed a higher capacity of arsenic methylation than their men counterparts did. These findings were compatible with some previous studies (Hopenhayn-Rich et al., 1996b; Hsueh et al., 1998), but in contrary to others (Chiou et al., 1997a; Kurttio et al., 1998).

Cigarette smokers had significantly higher urinary total arsenic and MMA^V percentage and lower SMI than non-smokers had (Table 1), implicating that smoking could exert an effect on the second methylation phase. This observation was also in conform to the study by Hopenhayn-Rich et al. (1996b) and a previous study in northeast Taiwan (Hsu et al., 1997). It is possible that some chemicals in cigarettes compete for some of the enzymes or co-factors involved in the methylation processes, particularly those involved in the second methylation phase. However, the effect of smoking was observed only in univariate analyses (Table 1) and not in multiple linear regression analyses (data not shown). These results also suggested that some other factors (for example, male sex) might have partly explained the effect of smoking on arsenic methylation capacity in our study.

In the present study, which was performed in the southwest coast of Taiwan, the MMA^V percentage (Table 1) was much lower than the previously reported 20–30% in the northeast coast of Taiwan (Chiou et al., 1997a). One of the possible explanations is that the exposure has been terminated for about 15–20 years when we conducted this study, but the exposure was continuous at the time when the study at the northeast coast of Taiwan was conducted. Because MMA^V percentage has been shown to increase with increasing dosage of exposure (Vahter, 1999b) and decrease after exposure dosage decreased (Hopenhayn-Rich et al.,

1996a), it was highly possible that the MMA^V would have been much higher than it had been shown in the study and the association with PVD would have been much stronger if our study had been carried out at the time when exposure was continuous.

The potential mechanisms of arsenic-induced atherosclerosis have been reviewed recently (Tseng, 2002; Simeonova and Luster, 2004). Although the mechanisms remain not completely elucidated, endothelial dysfunction, oxidative stress with impaired nitric oxide balance, and enhanced inflammatory and coagulating activities associated with arsenic exposure are potential explanations. Arsenic has been shown to induce endothelial dysfunction (Lee et al., 2003), peroxynitrite generation, and cyclooxygenase-2 protein expression in endothelial cells (Bunderson et al., 2002; Tsai et al., 2002). Arsenic also induces expression of genes coding for inflammatory mediators including IL-8 in human aortic endothelial cell (Simeonova et al., 2003). Some recent studies also demonstrated that arsenic induces antioxidative enzymes, including heme oxygenase-1, thioredoxin peroxidase-2, NADPH dehydrogenase, and glutathione *S*-transferase P subunit, suggesting the induction of oxidative stress by arsenic (Hirano et al., 2003). Our previous study demonstrated the existence of microcirculatory defects in seemingly normal subjects living in BFD-hyperendemic areas (Tseng et al., 1995). A recent in vitro study confirmed that exposure of human microvascular endothelial cells to arsenic resulted in a decrease of tissue-type plasminogen activator and an increase in plasminogen activator inhibitor type-1 expression as well as reduced fibrinolysis, which were not likely shown in macrovascular endothelial cells (Jiang et al., 2002). An endemic area of chronic arsenic poisoning and experimental animal studies elucidated a potential in vivo impairment of nitric oxide formation and oxidative stress caused by prolonged exposure to arsenate in the drinking water (Kumagai and Pi, 2004). Bunderson et al. (2004) reported that changes in specific inflammatory mediators such as leukotriene and prostacyclin are related to arsenic-induced atherosclerosis. The findings of the present study further provided evidence that, in addition to the above-mentioned possible mechanisms of arsenic-induced atherosclerosis, individual methylation capacity of inorganic arsenic could determine the susceptibility of PVD development in arsenic-exposed subjects.

Recently, the key metabolic intermediates, MMA^{III} and DMA^{III}, have been identified in human urine (Aposhian et al., 2000a, 2000b; Del Razo et al., 2001; Le et al., 2000a, 2000b; Mandal et al., 2001). Many studies also demonstrated that the trivalent methylated arsenic species are more toxic than the pre-methylated inorganic compounds. MMA^{III} and DMA^{III} could exhibit properties of inhibition on cysteine-containing enzymes (Lin et al., 2001; Styblo et al., 1997), cellular toxicity (Petrick et al., 2000), genotoxicity, and clastogenicity (Buratti et al., 1984; Mass et al., 2001; Zhong and Mass, 2001). In addition, MMA^{III} is a potent and irreversible inhibitor of an enzyme involved in cellular response to oxidative stress (Lin et al., 2001). The DNA damage induced by methylated trivalent arsenicals can either be direct (Mass et al., 2001) or mediated by the reactive oxygen species formed concomitantly with the oxidation of DMA^{III} to DMA^V (Nesnow et al., 2002). DMA^{III} can produce dimethylarsinic peroxyl radical and dimethylarsinic radical (Yamanaka et al., 2001). Arsenic is diabetogenic (Tseng, 2004; Tseng et al., 2000, 2002) and a recent study has clearly demonstrated that insulin-dependent glucose uptake by 3T3-L1 adipocytes is also significantly inhibited by trivalent arsenicals, either in inorganic form or in methylated form (Walton et al., 2004).

A recent study showed that MMA^{III} accumulated faster than other arsenic species in endothelial cells and were highly toxic (Hirano et al., 2004). However, the trivalent forms of methylated arsenic metabolites are not stable, and whether they can be detected depends on the conditions and temperature of sample storage and their concentrations in urine (Del Razo et al., 2001). We did not observe the presence of trivalent forms of methylated metabolites in our current study. One of the reasons was that the trivalent methylated forms were not known to be present and had not been identified from the urine at the time when our urinary samples were collected and analyzed. It is also possible that the concentrations of these trivalent methylated metabolites were too low to be detected in our samples, because MMA^{III} is more easily found in the urine after administration of sodium 2,3-dimercapto-1-propane sulfonate (DMPS), which assists the release of MMA^{III} from the body (Le et al., 2000b), and MMA^{III} would be more readily detected with higher arsenic exposure (Aposhian et al., 2000a, 2000b; Le et al., 2000a, 2000b). Our study subjects had stopped drinking artesian well water for about 15–20 years when their urine samples were collected, and they were not administered DMPS before we collected their urine. Therefore, it would not be too surprising for not detecting the trivalent methylated species in our samples. It has also been shown that the trivalent methylated arsenic species can be oxidized to the pentavalent forms after collection (Del Razo et al., 2001) and that DMPS can reduce pentavalent arsenicals to trivalent forms (Delnomdedieu et al., 1994). Furthermore, there is no known standard procedure to stabilize these trivalent methylated metabolites to serve the purpose of epidemiologic studies. Therefore, it will be difficult to use these metabolites as markers for the study of disease association at the present time. However, further investigation with focus on the association between these highly toxic species of arsenic metabolic intermediates and clinical diseases is worthwhile.

A study reported that arsenic species were stable for up to 2 months if urine samples were stored without any additives at temperatures of 4 and −20 °C; and some urinary samples were stable for a period of even up to 8 months (Feldmann et al., 1999). Le et al. (2000b) reported that approximately 60% of MMA^{III} and 95% DMA^{III} were oxidized to MMA^V

and DMA^V after the sample was stored at 4 °C for 2 weeks. Our previous data showed that arsenic species in human urine is stable for at least 6 months preserved at −20 °C (Chen et al., 2002). Because the assay of arsenic species was performed within 6 months after collection of the urinary samples and all samples were stored at a temperature of −20 °C, we believed that the arsenic species as measured in the present study should be reliable. Because MMA^V and DMA^V are generally considered as non-toxic, we cannot exclude the possibility that the higher MMA^V in the urine is a marker of higher MMA^{III} in the blood or inside the cells, where the injuries incurred by arsenic occur. Studies also showed that people with a lower MMA^V excretion in the urine tend to have a lower retention of arsenic (Vahter, 2002). This could also possibly explain why people with a lower MMA^V percentage tend to have a lower risk of developing arsenic-related diseases.

To take into account the uncertainty due to the change of urinary volume, some studies used urinary creatinine level for adjustment while expressing urinary arsenic concentration (Calderon et al., 1999; Del Razo et al., 1997; Hopenhayn-Rich et al., 1996b; Loffredo et al., 2003; Ma and Le, 1998; Tokunaga et al., 2002). However, this adjustment has been shown to be unnecessary in population studies (Hinwood et al., 2002). In the present study, urinary creatinine concentration was not measured and its adjustment could not be performed. However, except for urinary total arsenic, all of the other urinary arsenic parameters were expressed as ratios between arsenic species. These calculations have actually eliminated the potential influence of renal function or volume change because dividing the nominator and denominator simultaneously by a similar factor of urinary creatinine would not give a different ratio.

In conclusion, PVD risk in BFD-hyperendemic area in Taiwan is associated with a higher exposure dosage of arsenic and a lower capacity to methylate inorganic arsenic to DMA^V. These observations could explain partly why some subjects with a high exposure dosage would not develop clinical PVD. However, the association between PVD and the undetected trivalent forms of methylated metabolites in this study awaits further clarification.

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References

Andreae, M.O., 1977. Determination of arsenic species in natural waters. Anal. Chem. 49, 820–823.

Aposhian, H.V., Gurzau, E.S., Le, X.C., Gurzau, A., Healy, S.M., Lu, X., Ma, M., Yip, L., Zakharyan, R.A., Maiorino, R.M., Dart, R.C., Tircus, M.G., Gonzalez-Ramirez, D., Morgan, D.L., Avram, D., Aposhian, M.M., 2000a. Occurrence of monomethylarsonous acid in urine of humans exposed to inorganic arsenic. Chem. Res. Toxicol. 13, 693–697.

Aposhian, H.V., Zheng, B., Aposhian, M.M., Le, X.C., Cebrian, M.E., Cullen, W., Zakharyan, R.A., Ma, M., Dart, R.C., Cheng, Z., Andrewes, P., Yip, L., O'Malley, G.F., Maiorino, R.M., Van Voorhies, W., Healy, S.M., Titcomb, A., 2000b. DMPS-arsenic challenge test: II. Modulation of arsenic species, including monomethylarsonous acid (MMA(III)), excreted in human urine. Toxicol. Appl. Pharmacol. 165, 74–83.

Buchet, J.P., Lauwerys, R., Roels, H., 1980. Comparison of several methods for the determination of arsenic compounds in water and in urine. Their application for the study of arsenic metabolism and for the monitoring of workers exposed to arsenic. Int. Arch. Occup. Environ. Health 46, 11–29.

Bunderson, M., Coffin, J.D., Beall, H.D., 2002. Arsenic induces peroxynitrite generation and cyclooxygenase-2 protein expression in aortic endothelial cells: possible role in atherosclerosis. Toxicol. Appl. Pharmacol. 184, 11–18.

Bunderson, M., Brooks, D.M., Walker, D.L., Rosenfeld, M.E., Coffin, J.D., Beall, H.D., 2004. Arsenic exposure exacerbates atherosclerotic plaque formation and increases nitrotyrosine and leukotriene biosynthesis. Toxicol. Appl. Pharmacol. 201, 32–39.

Buratti, M., Calzaferri, G., Caravelli, G., Colombi, A., Maroni, M., Foa, V., 1984. Significance of arsenic metabolic forms in urine. Part I: chemical speciation. Int. J. Environ. Anal. Chem. 17, 25–34.

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

Chang, C.C., Ho, S.C., Tsai, S.S., Yang, C.Y., 2004. Ischemic heart disease mortality reduction in an arseniasis-endemic area in southwestern Taiwan after a switch in the tap-water supply system. J. Toxicol. Environ. Health, Part A 67, 1353–1361.

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.

Chen, Y.C., Amarasiriwardena, C.J., Hsueh, Y.M., Christiani, D.C., 2002. Stability of arsenic species and insoluble arsenic in human urine. Cancer Epidemiol., Biomarkers Prev. 11, 1427–1433.

Chen, Y.C., Guo, Y.L., Su, H.J., Hsueh, Y.M., Smith, T.J., Ryan, L.M., Lee, M.S., Chao, S.C., Lee, J.Y., Christiani, D.C., 2003a. Arsenic methylation and skin cancer risk in southwestern Taiwan. J. Occup. Environ. Med. 45, 241–248.

Chen, Y.C., Su, H.J., Guo, Y.L., Hsueh, Y.M., Smith, T.J., Ryan, L.M., Lee, M.S., Christiani, D.C., 2003b. Arsenic methylation and bladder cancer risk in Taiwan. Cancer Causes Control 14, 303–310.

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., Chu, T.H., Chen-Wu, C., Yang, M.H., Chen, C.J., 1997a. 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.

Chiou, H.Y., Huang, W.I., Su, C.L., Chang, S.F., Hsu, Y.H., Chen, C.J., 1997b. Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 28, 1717–1723.

Chowdhury, U.K., Rahman, M.M., Sengupta, M.K., Lodh, D., Chanda, C.R., Roy, S., Quamruzzaman, Q., Tokunaga, H., Ando, M., Chakraborti, D., 2003. Pattern of excretion of arsenic compounds [arsenite, arsenate, MMA(V), DMA(V)] in urine of children compared to adults from an arsenic exposed area in Bangladesh. J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng. 38, 87–113.

Delnomdedieu, M., Basti, M.M., Otvos, J.D., Thomas, D.J., 1994. Reduction and binding of arsenate and dimethylarsenate by glutathione: a multinuclear magnetic resonance study. Chem. Biol. Interact. 90, 139–155.

Del Razo, L.M., Garcia-Vargas, G.G., Vargas, H., Albores, A., Gonsebatt, M.E., Montero, R., Ostrosky-Wegman, P., Kelsh, M., Cebrian, M.E., 1997. Altered profile of urinary arsenic metabolites in adults with chronic arsenicism. A pilot study. Arch. Toxicol. 71, 211–217.

Del Razo, L.M., Styblo, M., Cullen, W.R., Thomas, D.J., 2001. Determination of trivalent methylated arsenicals in biological matrices. Toxicol. Appl. Pharmacol. 174, 282–293.

Engel, R.R., Hopenhaynrich, C., Receveur, O., Smith, A.H., 1994. Vascular effects of chronic arsenic exposure—a review. Epidemiol. Rev. 16, 184–209.

Feldmann, J., Lai, V.W., Cullen, W.R., Ma, M., Lu, X., Le, X.C., 1999. Sample preparation and storage can change arsenic speciation in human urine. Clin. Chem. 45, 1988–1997.

Gebel, T.W., 2002. Arsenic methylation is a process of detoxification through accelerated excretion. Int. J. Hyg. Environ. Health 205, 505–508.

Hinwood, A.L., Sim, M.R., de Klerk, N., Drummer, O., Gerostamoulos, J., Bastone, E.B., 2002. Are 24-hour urine samples and creatinine adjustment required for analysis of inorganic arsenic in urine in population studies? Environ. Res. 88, 219–224.

Hirano, S., Cui, X., Li, S., Kanno, S., Kobayashi, Y., Hayakawa, T., Shraim, A., 2003. Difference in uptake and toxicity of trivalent and pentavalent inorganic arsenic in rat heart microvessel endothelial cells. Arch. Toxicol. 77, 305–312.

Hirano, S., Kobayashi, Y., Cui, X., Kanno, S., Hayakawa, T., Shraim, A., 2004. The accumulation and toxicity of methylated arsenicals in endothelial cells: important roles of thiol compounds. Toxicol. Appl. Pharmacol. 198, 458–467.

Hopenhayn-Rich, C., Biggs, M.L., Kalman, D.A., Moore, L.E., Smith, A.H., 1996a. Arsenic methylation patterns before and after changing from high to lower concentrations of arsenic in drinking water. Environ. Health Perspect. 104, 1200–1207.

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

Hsueh, Y.M., Chiou, H.Y., Huang, Y.L., Wu, W.L., Huang, C.C., Yang, M.H., Lue, L.C., Chen, G.S., Chen, C.J., 1997. Serum beta-carotene level, arsenic methylation capability, and incidence of skin cancer. Cancer Epidemiol. Biomarkers Prev. 6, 589–596.

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, Part A 54, 431–444.

Hsu, K.H., Froines, J.R., Chen, C.J., 1997. Studies of arsenic ingestion from drinking-water in northeastern Taiwan: chemical speciation and urinary metabolites. In: Abernathy, C.O., Calderon, R.L., Chappell, W.R. (Eds.), Arsenic Exposure and Health Effects, vol. II. Chapman & Hall, New York, pp. 190–209.

Jiang, S.J., Lin, T.M., Wu, H.L., Han, H.S., Shi, G.Y., 2002. Decrease of fibrinolytic activity in human endothelial cells by arsenite. Thromb. Res. 105, 55–62.

Kalman, D.A., Hughes, J., van Belle, G., Burbacher, T., Bolgiano, D., Coble, K., Mottet, N.K., Polissar, L., 1990. The effect of variable environmental arsenic contamination on urinary concentrations of arsenic species. Environ. Health Perspect. 89, 145–151.

Kitchin, K.T., 2001. Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol. Appl. Pharmacol. 172, 249–261.

Kumagai, Y., Pi, J., 2004. Molecular basis for arsenic-induced alteration in nitric oxide production and oxidative stress: implication of endothelial dysfunction. Toxicol. Appl. Pharmacol. 198, 450–457.

Kuo, T.L., 1964. Arsenic Content of Artesian Well Water in Endemic Area of Chronic Arsenic Poisoning. Institute of Pathology, National Taiwan University, Taiwan, pp. 7–13.

Kurttio, P., Komulainen, H., Hakala, E., Kahelin, H., Pekkanen, J., 1998. Urinary excretion of arsenic species after exposure to arsenic present in drinking water. Arch. Environ. Contam. Toxicol. 34, 297–305.

Le, X.C., Lu, X., Ma, M., Cullen, W.R., Aposhian, H.V., Zheng, B., 2000a. Speciation of key arsenic metabolic intermediates in human urine. Anal. Chem. 72, 5172–5177.

Le, X.C., Ma, M., Cullen, W.R., Aposhian, H.V., Lu, X., Zheng, B., 2000b. Determination of monomethylarsonous acid, a key arsenic methylation intermediate, in human urine. Environ. Health Perspect. 108, 1015–1018.

Lee, M.Y., Jung, B.I., Chung, S.M., Bae, O.N., Lee, J.Y., Park, J.D., Yang, J.S., Lee, H., Chung, J.H., 2003. Arsenic-induced dysfunction in relaxation of blood vessels. Environ. Health Perspect. 111, 513–517.

Lin, S., Del Razo, L.M., Styblo, M., Wang, C., Cullen, W.R., Thomas, D.J., 2001. Arsenicals inhibit thioredoxin reductase in cultured rat hepatocytes. Chem. Res. Toxicol. 14, 305–311.

Loffredo, C.A., Aposhian, H.V., Cebrian, M.E., Yamauchi, H., Silbergeld, E.K., 2003. Variability in human metabolism of arsenic. Environ. Res. 92, 85–91.

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

Maki-Paakkanen, J., Kurttio, P., Paldy, A., Pekkanen, J., 1998. Association between the clastogenic effect in peripheral lymphocytes and human exposure to arsenic through drinking water. Environ. Mol. Mut. 32, 301–313.

Mandal, B.K., Ogra, Y., Suzuki, K.T., 2001. Identification of dimethylarsinous and monomethylarsonous acids in human urine of the arsenic-affected areas in West Bengal, India. Chem. Res. Toxicol. 14, 371–378.

Marafante, E., Vahter, M., Norin, H., Envall, J., Sandstrom, M., Christakopoulos, A., Ryhage, R., 1987. Biotransformation of dimethylarsinic acid in mouse, hamster and man. J. Appl. Toxicol. 7, 111–117.

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, 355–361.

Meza, M.M., Kopplin, M.J., Burgess, J.L., Gandolfi, A.J., 2004. Arsenic drinking water exposure and urinary excretion among adults in the Yaqui Valley, Sonora, Mexico. Environ. Res. 96, 119–126.

Murer, A.J., Abildtrup, A., Poulsen, O.M., Christensen, J.M., 1992. Effect of seafood consumption on the urinary level of total hydride-generating arsenic compounds, instability of arsenobetaine and arsenocholine. Analyst 117, 677–680.

Nesnow, S., Roop, B.C., Lambert, G., Kadiiska, M., Mason, R.P., Cullen, W.R., Mass, M.J., 2002. DNA damage induced by methylated trivalent arsenicals is mediated by reactive oxygen species. Chem. Res. Toxicol. 15, 1627–1634.

Nordstrom, D.K., 2002. Public health—Worldwide occurrences of arsenic in ground water. Science 296, 2143–2145.

Petrick, J.S., Ayala-Fierro, F., Cullen, W.R., Carter, D.E., Vasken, A.H., 2000. Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. Toxicol. Appl. Pharmacol. 163, 203–207.

Sakurai, T., Kojima, C., Ochiai, M., Ohta, T., Fujiwara, K., 2004. Evaluation of in vivo acute immunotoxicity of a major organic arsenic compound arsenobetaine in seafood. Int. Immunopharmacol. 4, 179–184.

Shraim, A., Sekaran, N.C., Anuradha, C.D., Hirano, S., 2002. Speciation of arsenic in tube-well water samples collected from West Bengal, India, by high-performance liquid chromatography-inductively

coupled plasma mass spectrometry. Appl. Organomet. Chem. 16, 202–209.
Simeonova, P.P., Luster, M.I., 2004. Arsenic and atherosclerosis. Toxicol. Appl. Pharmacol. 198, 444–449.
Simeonova, P.P., Hulderman, T., Harki, D., Luster, M.I., 2003. Arsenic exposure accelerates atherogenesis in apolipoprotein E−/−mice. Environ. Health Perspect. 111, 1744–1748.
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., Drobna, Z., Jaspers, I., Lin, S., Thomas, D.J., 2002. The role of biomethylation in toxicity and carcinogenicity of arsenic: a research update. Environ. Health Perspect. 110 (Suppl 5), 767–771.
Thomas, D.J., Styblo, M., Lin, S., 2001. The cellular metabolism and systemic toxicity of arsenic. Toxicol. Appl. Pharmacol. 176, 127–144.
Thomas, D.J., Waters, S.B., Styblo, M., 2004. Elucidating the pathway for arsenic methylation. Toxicol. Appl. Pharmacol. 198, 319–326.
Thompson, D.J., 1993. A chemical hypothesis for arsenic methylation in mammals. Chem. Biol. Interact. 88, 14–89.
Tokunaga, H., Roychowdhury, T., Chandraskaran, N., Uchino, T., Ando, M., 2002. Urinary arsenic species in an arsenic-affected area of West Bengal. India. Appl. Organomet. Chem. 16, 406–414.
Tsai, S.H., Liang, Y.C., Chen, L., Ho, F.M., Hsieh, M.S., Lin, J.K., 2002. Arsenite stimulates cyclooxygenase-2 expression through activating IkappaB kinase and nuclear factor kappaB in primary and ECV304 endothelial cells. J. Cell. Biochem. 84, 750–758.
Tseng, W.P., 1989. Blackfoot disease in Taiwan: a 30-year follow-up study. Angiology 40, 547–558.
Tseng, C.H., 2002. An overview on peripheral vascular disease in blackfoot disease-hyperendemic villages in Taiwan. Angiology 53, 529–537.
Tseng, C.H., 2004. The potential biological mechanisms of arsenic-induced diabetes mellitus. Toxicol. Appl. Pharmacol. 197, 67–83.
Tseng, C.H., Chen, C.J., Lin, B.J., Tai, T.Y., 1994. Abnormal response of ankle pressure after exercise in seemingly normal subjects living in blackfoot disease-hyperendemic villages in Taiwan. Vasc. Surg. 28, 607–617.
Tseng, C.H., Tai, T.Y., Lin, B.J., Chen, C.J., 1995. Abnormal peripheral microcirculation in seemingly normal subjects living in blackfoot disease-hyperendemic villages in Taiwan. Int. J. Microcirc. 5, 21–27.
Tseng, C.H., Chong, C.K., Chen, C.J., Tai, T.Y., 1996. Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. Atherosclerosis 120, 125–133.
Tseng, C.H., Chong, C.K., Chen, C.J., Tai, T.Y., 1997. Lipid profile and peripheral vascular disease in arseniasis-hyperendemic villages in Taiwan. Angiology 48, 321–335.
Tseng, C.H., Tai, T.Y., Chong, C.K., Tseng, C.P., Lai, M.S., Lin, B.J., Chiou, H.Y., Hsueh, Y.M., Hsu, K.H., Chen, C.J., 2000. Long-term arsenic exposure and incidence of non-insulin-dependent diabetes mellitus: a cohort study in arseniasis-hyperendemic villages in Taiwan. Environ. Health Perspect. 108, 847–851.
Tseng, C.H., Tseng, C.P., Chiou, H.Y., Hsueh, Y.M., Chong, C.K., Chen, C.J., 2002. Epidemiologic evidence of diabetogenic effect of arsenic. Toxicol. Lett. 133, 69–76.
Tseng, C.H., Chong, C.K., Tseng, C.P., Hsueh, Y.M., Chiou, H.Y., Tseng, C.C., Chen, C.J., 2003. Long-term arsenic exposure and ischemic heart disease in arseniasis-hyperendemic villages in Taiwan. Toxicol. Lett. 137, 15–21.
Vahter, M., 1999a. Variation in human metabolism of arsenic. In: Abernathy, C.O., Calderon, R.L., Chappell, W.R. (Eds.), Arsenic Exposure and Health Effects. Elsevier, New York, pp. 267–279.
Vahter, M., 1999b. Methylation of inorganic arsenic in different mammalian species and population groups. Sci. Prog. 82 (Pt. 1), 69–88.
Vahter, M., 2002. Mechanisms of arsenic biotransformation. Toxicology 181–182, 211–217.
Walton, F.S., Harmon, A.W., Paul, D.S., Drobna, Z., Patel, Y.M., Styblo, M., 2004. Inhibition of insulin-dependent glucose uptake by trivalent arsenicals: possible mechanism of arsenic-induced diabetes. Toxicol. Appl. Pharmacol. 198, 424–433.
WHO, 1980. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemical to Humans: Some Metal and Metallic Compounds. World Health Organization, Lyon, IARC.
Wu, H.Y., Chen, K.P., Tseng, W.P., Hsu, C.L., 1961. Epidemiologic studies on Blackfoot disease: I. Prevalence and incidence of the disease by age, sex, occupation and geographical distribution. Mem. Coll. Med. Natl. Taiwan Univ. 7, 33–50.
Yamanaka, K., Takabayashi, F., Mizoi, M., An, Y., Hasegawa, A., Okada, S., 2001. Oral exposure of dimethylarsinic acid, a main metabolite of inorganic arsenics, in mice leads to an increase in 8-Oxo-2′-deoxyguanosine level, specifically in the target organs for arsenic carcinogenesis. Biochem. Biophys. Res. Commun. 287, 66–70.
Yamauchi, H., Fowler, B.A., 1994. Toxicity and metabolism of inorganic and methylated arsenicals. In: Nriagu, J.O. (Ed.), Arsenic in The Environment, Part 2: Human Health and Ecosystem Effects. John Wiley and Sons, New York, pp. 33–53.
Yu, R.C., Hsu, K.H., Chen, C.J., Froines, J.R., 2000. Arsenic methylation capacity and skin cancer. Cancer Epidemiol., Biomarkers Prev. 9, 1259–1262.
Zhong, C.X., Mass, M.J., 2001. Both hypomethylation and hypermethylation of DNA associated with arsenite exposure in cultures of human cells identified by methylation-sensitive arbitrarily-primed PCR. Toxicol. Lett. 122, 223–234.