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Effects of nutritional measures on toenail arsenic concentration as a biomarker of arsenic exposure

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Abstract

Correlations between drinking water and toenail arsenic concentrations have been demonstrated in previous studies, yet factors that may modify the exposure–biomarker association have not been adequately assessed. Using data from 500 controls enrolled in a bladder cancer study underway in Michigan, USA, the effects of demographic characteristics and nutritional measures on the biomarker response were evaluated. Drinking water and toenail samples were collected during a home visit and analyzed for arsenic and other elements. Participants reported dietary supplement intake habits and provided demographic data. Arsenic concentrations of drinking water and toenail samples were positively correlated. Of the nutritional measures evaluated, toenail iron concentration was a significant modifier of the exposure–biomarker association. No demographic characteristics or general measures of dietary intake affected the biomarker response. The results presented herein are critical for biomarker validation and prove promising for sound application of the arsenic toenail biomarker to future epidemiological investigations.

Keywords: *arsenic, toenail, exposure biomarker, drinking water, diet*

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Introduction

Toenail arsenic concentrations have been used as a biomarker of arsenic exposure in epidemiological studies (Beane-Freeman et al. 2004, Karagas et al. 2004, Michaud et al. 2004) because following exposure, arsenic binds to keratin proteins in nails predominantly as inorganic arsenic (Hopps 1977, NRC 1999, Mandal et al. 2003). The potential of the toenail element concentration to provide an integrated account of multiple exposure pathways and to reflect exposures occurring over a period of several months lends promise to epidemiological studies aiming to reduce exposure measurement error, an issue that complicates studies investigating health effects associated with low-level arsenic exposure (Cantor 2001). The ability to minimize

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such exposure misclassification, however, is dependent on the ability of the biomarker to reflect accurately exposure across comparison groups.

Researchers have reported that arsenic concentrations in drinking water and toenails are well correlated, suggesting that toenail arsenic concentration may be useful as a biomarker of arsenic exposure (Karagas et al. 2000, Hinwood et al. 2003, Schmitt et al. 2005). Recent work has moved beyond these findings to demonstrate that toenail arsenic concentrations reflect arsenic intake from water consumption (Slotnick et al. 2007), therefore adding validity to the biomarker. Validation of an exposure biomarker is, however, an iterative process involving many steps (Schulte 2001); one such step includes an understanding of modifiers of the exposure–biomarker relationship (Dor et al. 1999, Slotnick & Nriagu 2006a).

Selenium has been studied extensively for its affect on arsenic and is perhaps the element most likely to interact with arsenic and therefore affect the biomarker response. Arsenic and selenium are both metalloids, and have been known to interact metabolically. Although most evidence of interactions has been generated from animal studies (Levander 1977, Zeng et al. 2005), the findings have been substantiated by human studies conducted on both the cellular (Zeng 2001) and population (Yang et al. 2002) levels. Several metabolic mechanisms have been proposed to explain the interaction, yet the underlying process is unclear. The proposed mechanisms, reviewed recently by Zeng et al. (2005) include: bilary excretion of an As–Se compound (seleno-bis (S-glutathionyl) arsinium ion [$(GS)_2AsSe)^-$]), direct interaction in aqueous solutions, effects on zinc finger protein functions, interactions in cellular signalling pathways and antagonistic effects on methylation processes. Therefore, arsenic and selenium have the potential to interact both directly and indirectly through many different mechanisms. Perhaps of most relevance to the modification of the exposure–biomarker relationship, however, are direct interactions between arsenic and selenium which may be more likely to explain competition for binding sites in proteins incorporated into the nail. Selenium intake may modify incorporation of arsenic into tissues (Salbe et al. 1993, Yang et al. 2002, Miyazaki et al. 2005), and subsequently modify the exposure–biomarker relationship.

It is generally theorized that dietary intake of antioxidants such as vitamin E and vitamin C may negate arsenic-induced health effects by protecting against oxidative stress (Karasavvas et al. 2005, Ramanathan et al. 2005, Verret et al. 2005), but the role of these vitamins on the toenail arsenic biomarker has not been adequately investigated. There also is evidence from animal and human studies that other dietary factors, including body mass index (BMI), calcium, animal protein, folate, fibre and various vitamins and minerals, may affect arsenic metabolism and excretion (Milton et al. 2004, Mitra et al. 2004, Steinmaus et al. 2005, Verret et al. 2005). Specifically, rabbits fed diets low in choline, methionine and protein and then treated with arsenite exhibited a decrease in urinary DMA and an increase in total arsenic retention in liver and lung tissues when compared with rabbits fed a normal diet (Vahter & Marafante 1987); it is likely that this is a result of suppression of transmethylation reactions, which also might lead to an increase in retention of inorganic arsenic (Vahter & Marafante 1987). Similar effects of diet on arsenic metabolism have been explored recently in human populations. In a population-based study conducted in the western United States, dietary intake was associated with changes in arsenic methylation (Steinmaus et al. 2005). In particular, participants with low intake of iron and zinc excreted higher percentages of arsenic as MMA and a lower percentage as DMA than

subjects with the highest levels of intake (Steinmaus et al. 2005). Increasing evidence of the ability of diet, and vitamin and mineral intake in particular, to modify arsenic metabolism and the exposure–disease pathway supports the need to evaluate the influence of these factors on the exposure–biomarker response.

Although there is some evidence that age, gender and smoking may affect arsenic metabolism (NRC 1999), little evidence exists regarding the ability of various disease risk factors to affect the exposure–biomarker association, particularly for individuals exposed to levels of arsenic in drinking water <100 μg l^{-1}. An understanding of how different co-existing disease risk factors affect the exposure–biomarker association will assist in sound application of the toenail arsenic concentration biomarker to epidemiological studies.

This study aims to explore whether BMI, multivitamin or single vitamin (vitamins C and E) intake and mineral intake (Se, Zn, Ca, Fe) modify the relationship between drinking water arsenic concentration and toenail arsenic concentration, and therefore affect universal applicability of the biomarker. A secondary aim is to investigate the potential for common disease risk factors (e.g. age, smoking and gender) to affect the biomarker response.

Methods

Study population

Results are presented for controls ($n=500$) who participated in an ongoing population-based case–control study of arsenic exposure and bladder cancer in Michigan. Details on this study population have been published previously (Meliker et al. 2006, Slotnick et al. 2006, 2007). Briefly, recruitment was limited to persons who lived in an 11-county study area of Southeastern Michigan for at least 5 consecutive years prior to being contacted. Controls were selected from an age-weighted list using a random digit dialing procedure, and were matched to bladder cancer cases based on age (± 5 years), race and gender. Participants with a history of cancer were excluded, with the exception of non-melanoma skin cancers (squamous cell and basal cell carcinomas). All participants signed a written informed consent, and were assigned a random identification number to maintain confidentiality. A modest financial incentive was offered to participants, along with the results of their toenail tests. This study was approved by the University of Michigan Health Sciences Institutional Review Board (IRB-Health).

Interview data

All study participants completed a computer-aided telephone interview (CATI), conducted by trained interviewers at the Michigan Public Health Institute (MPHI). The questionnaire was derived from instruments used in recent studies of arsenic exposure in the USA (Steinmaus et al. 2003, Karagas et al. 2004, Ayotte et al. 2006). Participants self-reported number of 8-oz glasses of plain drinking water and beverages made with tap water consumed during an average month in the past year. Data on demographics, smoking habits, and use of single and multiple vitamin supplements were also obtained through the telephone interview. Specifically, participants were asked about regular (at least 4 times per week) consumption of single and multiple vitamin supplements. In addition, participants were asked if they

regularly take zinc, iron, selenium or any other dietary supplement. If an 'other' dietary supplement was taken, subjects were asked to specify the type of supplement taken. Current smoker was defined as currently smoking at least one cigarette per day for 6 months or longer.

Participants completed a home visit subsequent to the telephone interview. During the home visit, a subset of participants ($n = 231$) were asked again if they regularly (at least 4 times per week) consume a dietary supplement. For study efficiency, not all participants were asked to provide these detailed data; the subset did not differ from the total population with respect to the demographic characteristics presented in Table I (results not shown). Subjects reporting regular consumption of a dietary supplement were asked to provide the supplement container; supplement type, brand and dose were recorded by the interviewers. If the supplement was a multivitamin, the dose of each vitamin listed was recorded. Participants were also asked to report the number of supplement pills taken per day. Data from the telephone interview were compared with data collected from the home visit; when an unexplained discrepancy occurred, data from the home visit were used in analyses. From these data, daily vitamin or mineral dose was calculated for 231 participants.

Table I. Demographic characteristics for participants providing toenail samples ($n = 488$) compared with non-responders ($n = 12$).

	Participants providing samples		Non-responders	
Population characteristics	*n*	%	*n*	%
Age (years)				
≤50	54	11.1	1	8.3
51–64	163	33.4	5	41.7
65–69	107	21.9	1	8.3
≥70	164	33.6	5	41.7
Gender				
Male	384	78.7	8	66.7
Female	104	21.3	4	33.3
Race				
Black	12	2.5	2	16.7
White	440	90.2	9	75.0
Other	29	5.9	1	8.3
Not reported	7	1.4	0	0.0
Current smoker				
Yes	37	7.6	1	8.3
No	449	92.0	9	75.0
Not reported	2	0.4	2	16.7
Body mass index				
<25	180	36.9	3	25.0
25–30	236	48.4	5	41.7
≥30	64	13.1	4	33.3
Not reported	8	1.6	0	0.0
Drinking water source				
Public supply	260	53.3	7	58.3
Private well	208	42.6	3	25.0
Bottled	14	2.9	1	8.3
Not reported	6	1.2	1	8.3

Collection and preparation of toenail samples for analysis

Subjects were mailed a packet with materials and instructions for clipping their toenails upon completion of the telephone interview. Using the stainless steel clippers provided, participants were asked to collect clippings from all 10 toenails, if possible, after bathing or showering. Participants also recorded the date clipped. Study interviewers collected the toenail clippings during the home visit. Participants who were unable to provide a sample during the home visit were given a stamped envelope and asked to mail their sample to the researchers at a later date. Samples were obtained from 488 (97.6%) participants.

Following collection, toenail samples were washed and digested following modification of protocol reported in previous studies (Das et al. 1995, Samanta et al. 1999, Karagas et al. 2000). The methods have been previously described in detail (Slotnick et al. 2007). Briefly, samples were cleaned using acetone and water, dried overnight in a 60°C oven, cooled to room temperature, weighed and then digested with Optima HNO_3 (Fisher Chemical, Pittsburgh, PA, USA) and Suprapur H_2O_2 (Merck & Co., Whitehouse Station, NJ, USA). The final volume was diluted to 3 ml using Milli-Q water. For quality control purposes, digestion batches included reagent blanks and certified reference materials (NIST 1577b, Bovine Liver and NCSZC 81002, Trace Elements in Human Hair). To gain maximum recovery for Zn, all samples were diluted 1:10 and re-run; the diluted measurements were used as the final concentration for Zn. Diluted recoveries for other elements were compared with the concentrated recoveries to verify the absence of matrix interferences. Samples divided in half prior to washing or digestion were analyzed as replicates. For each batch, blank values were averaged and subtracted from the result to obtain a final concentration ($\mu g\ l^{-1}$). All values were then converted to $\mu g\ g^{-1}$ dry weight.

Collection of drinking water samples

Interviewers collected drinking water samples from all participants during the home visit, as previously described (Slotnick et al. 2006). The participant was asked to identify the primary source of drinking water. After running the tap for 2 min, samples were collected in 60 ml low-density polyethylene (LDPE) bottles, previously acid-washed for determination of trace metals (Nriagu et al. 1993). Samples were stored on ice in transit, acidified with 100 µl trace-metal grade HNO_3 (Fisher Chemical) in the lab, and refrigerated until analysis. To minimize contamination, non-powdered, vinyl gloves were worn to handle samples, and sample bottles were stored in plastic zippered bags both before and after sample collection. One field blank and one field replicate were collected each day for quality control purposes, resulting in blanks and replicates for 15% of the drinking water samples analyzed. Blanks consisted of a Milli-Q water sample transferred into a sample bottle at the field site. An average blank value was calculated for each analysis batch and subtracted from the concentration of each sample. If no sample was collected for participants drinking bottled water, an average of the bottled water values analyzed was assigned ($0.2\ \mu g\ l^{-1}$), although bottled water was listed as the primary drinking water source for less than 1% of participants. Multiple samples were collected and results were averaged if participants indicated drinking more than one type of water at home (e.g. bottled water and tap water).

Instrumental analysis

Toenail samples were analyzed for total As, Ca, Fe, Se and Zn and drinking water samples were analyzed for total As at the University of Michigan, School of Public Health using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent Technologies Model 7500c). The Agilent 7500c is equipped with a collision cell to minimize spectral interferences. Calibration standards were prepared immediately prior to each analysis by dilution of the Agilent Multi-Element Calibration Standard in 5% trace-metal grade HNO_3 (Fisher Chemical). The calibration was validated using NIST SRM 1640, Trace Elements in Natural Water; recovery for the SRM 1640 was always within 10% of the certified value. The method detection limit (MDL) for water was calculated as being three times the standard deviation of the calibration standard diluted to 50 ng l^{-1}, and varied by analysis batch. The average MDL for arsenic was calculated as 0.02 μg l^{-1} ($n = 17$); a value of one-half the average MDL (0.01 μg l^{-1}) was assigned for water samples below detection limit (BDL).

The instrument detection limit (IDL) for the toenail samples (equal to the MDL for water) was calculated as being three times the standard deviation of the calibration standard diluted to 50 ng l^{-1} for As, Se and Zn and 5 μg l^{-1} for Ca and Fe, and varied by analysis batch. The average MDL was calculated for toenails using the IDL and the average toenail weight (0.1320 g) (Table II). A value of one-half the MDL was assigned for values below the detection limit (BDL).

Statistical analyses

Intake of arsenic from drinking water at home (including consumption of plain water and beverages made with tap water) was calculated using intake estimates and drinking water arsenic concentration (glasses of water (litre/day) × drinking water arsenic concentration (μg l^{-1}) = arsenic intake (μg/day)). Multiple linear regression analyses using interaction terms were employed to test for modification of the relationship between toenail arsenic concentration and drinking water arsenic concentration and between toenail arsenic concentration and arsenic intake from drinking water. Potential modifiers considered were gender, age, current smoker (yes/no), multivitamin or single vitamin use (yes/no), specific single vitamin supplement

Table II. Recoveries and MDLs for elements analyzed in toenail samples.

	NCSZC 81002 (human hair)		NIST 1577b (bovine liver)				
Element	Average % recovery (SD)	*n*	Average % recovery (SD)	*n*	Average IDL (μg l^{-1})	Average MDL[a] (toenails) (μg g^{-1})	No. of toenail samples below MDL
As	101.9 (18.4)	39	102.3 (14.0)	39	0.04	0.001	0
Ca	n/a[b]	n/a[b]	93.2 (12.1)	39	4.42	0.087	1
Fe	93.5 (11.2)	39	102.2 (8.9)	39	2.81	0.054	1
Zn	90.34 (4.9)[c]	10[c]	99.3 (15.1)[c]	18[c]	0.41	0.008	14
Se	111.8 (31.5)	39	105.5 (27.3)	39	0.06	0.001	0

[a]Calculated using the average toenail weight (0.1320 g, $n = 899$); [b]the standard reference material is not certified for this element; [c]results of diluted (1:10) samples.

use and doses, and Se, Zn, Fe and Ca toenail element concentrations. Modifiers were assessed based on the following models:

$$\log_{10}\mathrm{TAS}_i = \beta_0 + \beta_1(\log_{10}\mathrm{WAs}_i) + \beta2(\log_{10}\mathrm{M}_i) + \beta3(\log_{10}\mathrm{WAs}_i \times \log_{10}\mathrm{M}_i) + \varepsilon_i$$

and

$$\log_{10}\mathrm{TAS}_i = \beta_0 + \beta_1(\log_{10}\mathrm{IAs}_i) + \beta2(\log_{10}\mathrm{M}_i) + \beta3(\log_{10}\mathrm{IAs}_i \times \log_{10}\mathrm{M}_i) + \varepsilon_i$$

where WAs = drinking water arsenic concentration and IAs = drinking water arsenic intake, and M is a generic term representing the modifying variable of interest (e.g. age, gender, etc.) (Table III).

Each modifier was also considered as an independent predictor of toenail arsenic concentration using simple linear regression, and Pearson correlation coefficients were calculated to evaluate correlations between supplement doses of Se, Zn, Fe and Ca and toenail concentrations of these elements. Histograms and normal probability plots demonstrated that data were log normally distributed (results not shown). Therefore, values for all continuous variables were $\log_{10}$ transformed prior to analyses. Delta-Betas (DFBETAS) were calculated, using a criteria of $\Delta\beta_{j(i)} > 2/(n^{1/2})$, to identify

Table III. Modifiers of the exposure–biomarker relationship by exposure estimate: results from multiple linear regression with interaction terms.

	Arsenic concentration ($\mu g\ l^{-1}$)		Arsenic intake (μg daily)	
Interaction variable	Drinking water parameter ($_1$)	Interaction parameter ($_3$)	Arsenic intake parameter ($_1$)	Interaction parameter ($_3$)
Demographics				
Age (years)	0.293	−0.001	0.372	−0.003
Gender (male/female)	0.198	0.028	0.161	0.033
Current smoker (yes/no)	0.189	0.081	0.154	0.130
Body mass index	0.224	−0.008	0.024	0.044
Dietary supplement use				
Any multivitamin	0.233	−0.053	0.219	−0.078
Any single supplement	0.194	0.015	0.185	−0.031
Single zinc supplement	0.194	0.041	0.163	0.037
Single iron supplement	0.200	0.001	0.172	-0.028
Single selenium supplement	0.188	0.099	0.155	0.107
Single vitamin C supplement	0.220	−0.073	0.194	−0.088
Single vitamin E supplement	0.212	−0.041	0.194	−0.089
Dietary supplement dose				
Vitamin C	0.283	−0.017	0.187	−0.013
Vitamin E	0.405	−0.047	0.312	−0.030
Zinc	0.419	−0.067	0.374	−0.060
Selenium	0.365	−0.042	0.295	−0.028
Iron	0.526	−0.065	0.480	0.073
Calcium	0.244	−0.009	0.301	−0.022
Toenail concentration				
Selenium	0.177	−0.044	0.145	−0.048
Zinc	0.295	−0.021	0.265	−0.022
Iron	0.328	−0.041[a]	0.305	−0.041[a]
Calcium	0.036	0.0249	0.058	0.017

[a]Interaction term significant at $\alpha = 0.05$ (p-values = 0.0132 and 0.0063 for drinking water arsenic and arsenic intake, respectively).

influential data points. All statistical analyses were run using SAS® statistical software, version 8 (SAS Institute Inc, Cary, NC, USA).

Results

Toenail and drinking water samples were provided by 488 (97.6%) of the participants. Demographic characteristics did not differ markedly for participants providing toenail samples when compared with those unable or unwilling to provide samples (Table I). The drinking water and toenail data for this population have been published previously (Slotnick et al. 2007).

Of the drinking water samples analyzed for arsenic, 39 were below the MDL. Both field and lab replicate drinking water samples were in close agreement with the primary samples ($r=0.995$, $n=172$, $p<0.0001$ and $r=0.999$, $n=11$, $p<0.0001$, respectively). Blank values were low, indicating that contamination of the samples was minimal; 126 of 162 (78%) blank samples were BDL, while arsenic concentrations in the remaining blanks were below 0.20 $\mu g\, l^{-1}$. For samples with concentrations greater than 50 $\mu g\, l^{-1}$, high correlation was observed between diluted (1:10) and concentrated samples ($R^2=0.998$, $n=6$); these results suggest that spectral interference did not bias the results. Drinking water arsenic concentration ranged from BDL to 99.3 $\mu g\, l^{-1}$, with 8.4% of samples exceeding the US Environmental Protection Agency (EPA) maximum contaminant level of 10 $\mu g\, l^{-1}$. The geometric mean for drinking water arsenic concentrations was 0.55 $\mu g\, l^{-1}$ (SD 5.8 $\mu g\, l^{-1}$, $n=500$).

No toenail samples were below detection level for arsenic (Table II). Average recoveries were within 12% of the certified value for NCSZC 81002 (human hair) and the indicative value for NIST 1577b (bovine liver) (Table II). Replicate samples were in good agreement with the primary samples ($r=0.80$, $n=14$, $p=0.001$). Blank values were low, with 92% (147/160) BDL; blank values above the IDL averaged 0.08 $\mu g\, l^{-1}$. Toenail arsenic concentration (TAs) ranged from 0.003 to 1.26 $\mu g\, g^{-1}$, and the geometric mean was equal to 0.07 $\mu g\, g^{-1}$ (SD 2.33 $\mu g\, g^{-1}$, $n=488$). Toenail arsenic concentration is positively and significantly correlated with drinking water arsenic concentration in this population; details of this association have been previously described (Slotnick et al. 2007). For the 488 participants described herein, a simple linear regression reveals drinking water arsenic concentration is a significant predictor of toenail arsenic concentration ($R^2=0.15$, $p<0.001$). Vitamin dose data for Fe, Se, Zn and Ca were not significantly correlated with corresponding toenail element concentrations (results not shown), although this result may be limited by the available dose data.

Use of dietary supplements was common in this population, with 61.5% of participants reporting regular intake of multivitamin supplements, and over half of participants reporting regular consumption of a single dietary supplement (Table IV).

Selenium and vitamin C supplement use was a significant independent predictor of toenail arsenic concentration, as were selenium, zinc, iron and calcium toenail concentrations (Table V). Of the variables explored as modifiers of the biomarker response, toenail iron concentration (TFe) was a significant effect modifier (Table III).

The model R^2 value increased from 0.17 for the simple model to 0.33 for the model with an interaction term for drinking water arsenic concentration, and from 0.13 to

Table IV. Frequency of dietary supplement use amongst participants providing toenail samples ($n = 488$).

Type of dietary supplement	Participants reporting regular use[a] *n* (%)
Any multivitamin	300 (61.5)
Any single supplement	240 (49.1)
Single zinc supplement	75 (15.4)
Single iron supplement	50 (10.2)
Single selenium supplement	46 (9.4)
Single vitamin C supplement	106 (21.7)
Single vitamin E supplement	136 (27.9)

[a]At least 4 times per week.

0.18 when comparing the simple model and the model with an interaction term for arsenic intake from drinking water. The interaction parameter was negative, indicating that the increase in toenail arsenic concentration observed with increasing drinking water arsenic concentration (or intake) decreases as toenail iron concentration increases. These interaction terms were significant for $\alpha = 0.05$. DFBETAS revealed the possibility of one influential point in the model evaluating the effect of iron supplement intake on the biomarker response. When this data point was removed, however, the model results did not change.

Table V. Independent predictors of toenail arsenic concentration: results from simple linear regression.

Independent variable	Parameter estimate (β)	Model R^2
Demographics		
Age (years)	−0.004	0.0030
Gender (male/female)	0.047	0.0005
Current smoker (yes/no)	−0.067	0.0005
Body mass index	0.073	0.0001
Dietary supplement use (yes/no)		
Any multivitamin	0.017	0.0001
Any single supplement	0.117	0.0047
Single zinc supplement	0.139	0.0035
Single iron supplement	0.039	0.0002
Single selenium supplement	0.327*	0.0128
Single vitamin C supplement	0.193*	0.0088
Single vitamin E supplement	0.048	0.0007
Dietary supplement dose		
Vitamin C (mg/day)	0.056	0.0088
Vitamin E (IU/day)	0.013	0.0007
Zinc (mg/day)	0.186	0.0035
Selenium (μg/day)	0.143	0.0128
Iron (μg/day)	0.265	0.0002
Calcium (mg/day)	0.153	0.0020
Toenail concentration		
Selenium (μg g^{-1})	0.717*	0.2208
Zinc (μg g^{-1})	0.182*	0.0088
Iron (μg g^{-1})	0.313*	0.1743
Calcium (μg g^{-1})	0.381*	0.0613

*Significant at $\alpha = 0.05$.

Discussion

Overall, the results presented herein are promising for application of toenail arsenic concentration as a biomarker of internal exposure to arsenic for epidemiological studies. In the current study, the ability of the biomarker to measure exposure to arsenic was not influenced by population characteristics such as age, smoking, BMI, and gender (Table III). Factors that modify the exposure biomarker may affect accurate prediction of disease risk by introducing exposure misclassification that varies differentially with predictors of disease risk. Therefore, the null results presented here are encouraging for sound application of the biomarker to epidemiological investigations.

Dietary supplement use was prevalent in this population, with over half of the population consuming dietary supplements on a regular basis (Table IV). Of the dietary supplements evaluated, regular (at least four times per week) intake of selenium supplements and vitamin C supplements correlated with significantly higher toenail arsenic concentrations. Studies have explored the ability of selenium to affect arsenic concentrations in various tissues (e.g. hair, nails, blood) of humans and animals (and vice versa), although the findings are contrary to the results presented herein (Salbe et al. 1993, Miyazaki et al. 2005). For example, male rats fed selenium-enriched diets and dosed with 10 ppm sodium arsenite in drinking water experienced a decrease in hair, nail, red cell, plasma and kidney selenium concentrations (Salbe et al. 1993).

Additionally, results from a selenium-enriched yeast supplementation trial in farmers of Inner Mongolia, China indicate statistically significant decreases in arsenic in hair among those taking the selenium supplements when compared with the placebo group (Yang et al. 2002). Concentrations of arsenic in Inner Mongolia range from below detection limit to 1.8 mg l^{-1} (Mo et al. 2006). From these studies one might expect a negative association between selenium supplementation and toenail arsenic concentration. The results presented herein suggest otherwise, emphasizing the need for research on these metabolic interactions when arsenic exposures are low.

Although vitamin C may mediate arsenic toxicity through mechanisms related to oxidative stress (Karasavvas et al. 2005, Ramanathan et al. 2005, Verret et al. 2005), a search of the literature revealed no studies addressing direct metabolic interactions which may explain the higher toenail arsenic associated with vitamin C intake. Therefore, other explanations for the associations should be explored. For instance, arsenic has been found in significant levels in some herbal dietary supplements, but little information is available on arsenic contamination of other supplements such as vitamin C and selenium (Slotnick & Nriagu 2006b). Thus, while ingested amounts are likely to be minute, the possibility of vitamins contributing to daily arsenic intake cannot be ruled out. Therefore, it is critical to measure trace elements in supplements and to continue to evaluate the influence of supplements on toenail arsenic concentration and on the biomarker response.

Toenail selenium, zinc, iron and calcium levels were each significant independent predictors of toenail arsenic concentration, although these variables alone do not explain a large percentage of variability in the toenail arsenic levels (Table V). Toenail arsenic and selenium concentrations have been shown to be positively correlated in previous studies (Slotnick et al. 2005); no other known studies have investigated the influence of zinc, iron and calcium toenail concentrations on toenail arsenic levels. It is possible that a common exposure source may explain the positive correlations.

Drinking water is the primary source of inorganic arsenic exposure in the study area from which this population was recruited (Meliker et al. 2006). Pearson correlations between concentrations of the elements in the drinking water samples revealed that iron and arsenic were positively correlated ($r = 0.30$, $p < 0.001$), although arsenic and calcium and arsenic and selenium were negatively correlated ($r = -0.32$, $p < 0.001$ and $r = -0.43$, $p < 0.001$, respectively). Drinking water arsenic and zinc concentrations were not significantly correlated. Therefore, it is unlikely that drinking water concentration of the elements explains the inter-element correlations in the toenail samples. An alternative explanation may involve individual-level differences in nail proteins or structure leading some individuals to accumulate higher element concentration in general; this possible explanation needs further research.

Gender, smoking status, BMI and age were not significant predictors of toenail arsenic concentration in this population (Table V). Other studies have also reported that smoking, gender and BMI do not influence toenail arsenic concentration (Chiou et al. 1997, Karagas et al. 2000, Hinwood et al. 2003, Kile et al. 2005). The results for age, however, are inconsistent with our previous publication in which age was a weak predictor of toenail arsenic concentration (Slotnick et al. 2007). Other studies have also hinted that age might influence toenail arsenic concentration (Vance et al. 1988, Das et al. 1995, Karagas et al. 2000). Additionally, in Bangladesh, where drinking water arsenic concentrations ranged from <1 to 752 $\mu g\, l^{-1}$, age positively modified the ability of drinking water exposure to predict toenail arsenic concentration for a population aged 7–77 years (Kile et al. 2005). Given the conflicting results, if age does influence the biomarker response, it is likely that the effect is weak. It should be noted that participants in our study were controls who were frequency-matched to cases enrolled in a bladder cancer study. Therefore, the study population has a high proportion of white males (Table I), with an average age of 65 years (range: 18–90 years) for participants providing nail samples. The influence of age on the biomarker response might therefore be less pronounced in this population than in a population including children. Furthermore, it has been suggested that different ethnic groups may metabolize arsenic differently, resulting in differential concentrations of nail arsenic concentration (Brima et al. 2006). The limited diversity of the population described herein may affect broader application of our findings.

Of the potential modifiers examined, toenail iron concentration was a significant modifier of the relationship between exposure and the biomarker value (Table III). The fact that this result holds using both drinking water arsenic concentration and arsenic intake from drinking water is supportive of a true association. In a population-based study conducted in the western United States, participants with low intake of iron, as estimated from a dietary questionnaire and accompanying nutrient composition database, excreted higher percentages of arsenic as MMA and inorganic arsenic and a lower percentage as DMA than subjects with the highest levels of intake (Steinmaus et al. 2005). Therefore, increased iron intake might improve the ability to metabolize arsenic, thereby decreasing the amount of inorganic arsenic incorporated into the nail. While intriguing, these findings warrant further research to elucidate the processes through which arsenic is incorporated into the nail bed, and the effect that iron has on arsenic metabolism.

In an earlier study conducted in the United States, selenium status, as measured by concentrations in toenails, was not found to influence the association between total arsenic concentration in toenail and residential drinking water samples (Karagas et al.

2000). Our results are consistent with these findings. Toenails are a fairly good marker of recent selenium exposure, based on a number of studies demonstrating associations between known selenium intake and nail concentrations (Slotnick & Nriagu 2006a). Therefore, the available data on toenail selenium concentration are a strength of this study; it is not expected that improved dietary assessment of selenium intake would alter the findings.

It is possible that our inability to observe significant effects of other vitamins and minerals on the biomarker response is due in part to limited dietary exposure assessment. While in the USA individual supplements generally contain higher doses of individual vitamins than diet or multivitamin supplements (Jacobs et al. 2002), a more complete dietary assessment may be beneficial for evaluating vitamins as modifiers of the exposure–biomarker association. In particular, there is increasing evidence that folate status may affect arsenic metabolism, as the methylation pathway for arsenic is folate-dependent (Gamble et al. 2006). Although data were obtained from the current study population on folate supplement use, few (3%) participants reported regular intake of folate supplements. Additionally, while it is possible to consider multivitamin use to be a surrogate of dietary intake, composition of multivitamins varies widely (Yetley 2007) and exposure misclassification would probably be high. Unfortunately, folate concentration of multivitamins was not obtained in this study. Future investigations should consider the use of a more complete FFQ, in addition to questions on dietary supplement intake, to better assess dietary intake.

This study is also limited by the self-report nature of the questionnaire data. Errors in this type of data are inevitable, and may result in some exposure misclassification. This exposure misclassification might be influencing the discrepancies observed in estimated parameter values for different models (Table III), as there are likely inaccuracies in reported drinking water arsenic intake values. However, steps were taken to minimize such exposure misclassification for the vitamin intake data. In-person interviews provided verification of the telephone interview data, participants were asked to provide visual representation of the dietary supplements consumed, and composition of the dietary supplements was verified and recorded by interviewers.

Although participants in this study were part of a large-scale case–control study with a study population greater than many previously conducted studies of arsenic exposure and cancer risk (Bates et al. 1995, Kurttio et al. 1999, Chen et al. 2003, Steinmaus et al. 2003, Bates et al. 2004), the ability to detect associations may be limited by the number of study participants. In particular, not all participants regularly used dietary supplements, and information on dietary supplement dose was not available for all participants. Demographic characteristics did not differ for those individuals reporting data on dietary supplement dose when compared with all 500 controls (results not shown). Because this was the first study to evaluate modifiers of the toenail arsenic biomarker response outside age, epidemiological studies utilizing toenails as a biomarker of arsenic exposure should also consider evaluation of exposure–biomarker modifiers, particularly for low-level arsenic exposure, to verify the results presented herein.

The use of element nail data as biomarkers of dietary intake and exposure is a strength of this study; however, understanding of the ability of toenails to reflect accurately exposure to these elements is critical. In this study, Se, Fe, Zn and Ca toenail concentrations were not positively correlated with estimated element dose

from dietary supplement intake. Other sources of element exposure, such as dietary intake, probably contribute to toenail element concentration, however, and this null finding may be due in part to inaccuracies in estimating total element dietary intake. Element nail concentrations of zinc, iron and calcium have been measured in previous studies (Takagi et al. 1988, Garland et al. 1993, Chaodhary et al. 1995) and toenail concentrations of zinc have been used in several studies to assess zinc status (Rogers et al. 1991, Platz et al. 2002, Martin-Moreno et al. 2003, Vinceti et al. 2005), yet there are very limited data supporting the ability of human nails to reflect exposure to any of these elements (McKenzie 1979, Shaaban et al. 2005, Wilhelm et al. 1991). Steps similar to those necessary to validate nails as a marker of arsenic and selenium exposure (Slotnick & Nriagu 2006a) should be taken to investigate the ability of nails to accurately measure exposure to other elements. Nonetheless, our findings of limited modification of the biomarker response by demographic variables and nutritional factors are promising for use of toenails as a biomarker of arsenic exposure.

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References

ASTDR. 1999. Toxicological Profile for Arsenic. Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Research Triangle Park, NC, USA.

Ayotte JD, Baris D, Cantor KP, Colt J, Robinson GP, Lubin JH, Karagas M, Hoover RN, Fraumeni JF, Silverman DT. 2006. Bladder cancer mortality and private well use in New England: an ecological study. Journal of Epidemiology & Community Health 60:168–172.

Bates MN, Smith AH, Cantor KP. 1995. Case-control study of bladder cancer and arsenic in drinking water. American Journal of Epidemiology 141:523–529.

Bates MN, Rey OA, Biggs ML, Hopenhayn C, Moore LE, Kalman D, Steinmaus C, Smith AH. 2004. Case-control study of bladder cancer and exposure to arsenic in Argentina. American Journal of Epidemiology 159:381–389.

Beane-Freeman LE, Dennis LK, Lynch CF, Thorne PS, Just CL. 2004. Total arsenic content and cutaneous melanoma in Iowa. American Journal of Epidemiology 160:679–687.

Brima EI, Haris PI, Jenkins RO, Polya DA, Gault AG, Harrington CF. 2006. Understanding arsenic metabolism through a comparative study of arsenic levels in the urine, hair and fingernails of healthy volunteers from three unexposed ethnic groups in the United Kingdom. Toxicology & Applied Pharmacology 216:122–130.

Cantor KP. 2001. Invited commentary: arsenic and cancer of the urinary tract. American Journal of Epidemiology 153:419–421.

Chaodhary K, Ehmann WD, Rengan K, Markesbery WR. 1995. Trace element correlations with age and sex in human fingernails. Journal of Radioanalysis & Nuclear Chemistry 195:51–66.

Chen YC, Su HJJ, Guo YLL, Hsueh YM, Smith TJ, Ryan LM, Lee MS, Christiani DC. 2003. Arsenic methylation and bladder cancer risk in Taiwan. Cancer Causes Control 14:303–310.

Chiou HY, Huang WI, Su CL, Chang SF, Hsu YH, Chen CJ. 1997. Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 28:1717–1723.

Das D, Chatterjee A, Mandal BK, Samanta G, Chakraborti D, Chanda B. 1995. Arsenic in ground water in six districts of west Bengal, India: the biggest arsenic calamity in the world. Part 2. Arsenic concentration in drinking water, nails, urine, skin-scale and liver tissue (biopsy) of the affected people. Analyst 120:917–924.

Dor, F, Dab, W, Empereur-Bissonnet P, Zmirou D. 1999. Validity of biomarkers in environmental health studies: the case of PAHs and Benzene. Critical Reviews in Toxicology 29:129–168.

Gamble MV, Liu Z, Ahsan H, Pilsner JR, Ilievski V, Slavkovich V, Parvez F, Chen Y, Levy D, Factor-Litvak P, Graziano JH. 2006. Folate and arsenic metabolism: a double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. American Journal of Clinical Nutrition 84:1093–1101.

Garland M, Morris JS, Rosner BA, Stamfer MJ, Spate VL, Baskett CJ, Willett WC, Hunter DJ. 1993. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. Cancer, Epidemiology, Biomarkers & Prevention 2:493–497.

Hinwood AL, Sim MR, Jolley D, de Klerk N, Bastone EB, Gerostamoulos J, Drummer OH. 2003. Hair and toenail arsenic concentrations of residents living in areas with high environmental arsenic concentrations. Environmental Health Perspectives 111:187–193.

Hopps HC. 1977. The biologic basis for using hair and nail for analyses of trace elements. The Science of the Total Environment 7:71–89.

Jacobs EJ, Henion AK, Briggs PJ, Connell CJ, McCullough ML, Jonas CR, Rodriguez C, Calle EE, Thun MJ. 2002. Vitamin C and vitamin E supplement use and bladder cancer mortality in a large cohort of US men and women. American Journal of Epidemiology 156:1002–1010.

Karagas MR, Tosteson TD, Blum J. 2000. Measurement of low levels of arsenic exposure: a comparison of water and toenail concentrations. American Journal of Epidemiology 152:84–90.

Karagas MR, Tosteson TD, Morris JS, Demidenko E, Mott LA, Heaney J, Schned A. 2004. Incidence of transitional cell carcinoma of the bladder and arsenic exposure in New Hampshire. Cancer Causes Control 15:465–472.

Karasavvas N, Carcamo JM, Stratis G, Golde DW. 2005. Vitamin C protects HL60 and U266 cells from arsenic toxicity. Blood 105:4004–4012.

Kile ML, Houseman EA, Rodrigues E, Smith TJ, Quamruzzaman Q, Rahman M, Mahiuddin G, Su L, Christiani DC. 2005. Toenail arsenic concentrations, GSTT1 gene polymorphisms and arsenic exposure from drinking water. Cancer, Epidemiology, Biomarkers & Prevention 14:2419–2426.

Kurttio P, Pukkala E, Kahelin H, Auvinen A, Pekkanen J. 1999. Arsenic concentrations in well water and risk of bladder and kidney cancer in Finland. Environmental Health Perspectives 107:705–710.

Levander OA. 1977. Metabolic interrelationships between arsenic and selenium. Environmental Health Perspectives 19:159–164.

Mandal BK, Ogra Y, Suzuki KT. 2003. Speciation of arsenic in human nail and hair from arsenic-affected area by HPLC-inductively coupled argon plasma mass spectrometry. Toxicology & Applied Pharmacology 189:73–83.

Martin-Moreno JM, Gorgojo L, Riemersma RA, Gomez-Aracena J, Kark JD, Guillen J, Jimenez J, Ringstad JJ, Fernandez-Crehuet J, Bode P, Kok FJ. 2003. Myocardial infarction risk in relation to zinc concentration in toenails. British Journal of Nutrition 89:673–678.

McKenzie JM. 1979. Content of zinc in serum, urine, hair and toenails of New Zealand adults. American Journal of Clinical Nutrition 32:570–579.

Meliker JR, Franzblau A, Slotnick MJ, Nriagu JO. 2006. Major contributors to inorganic arsenic intake in Southeastern Michigan. International Journal of Hygiene & Environmental Health 209:399–411.

Michaud DS, Wright M, Cantor KP, Taylor PR, Virtamo J, Albanes D. 2004. Arsenic concentrations in prediagnostic toenails and the risk of bladder cancer in a cohort study of male smokers. American Journal of Epidemiology 160:853–859.

Milton AH, Hasan Z, Shahidullah SM, Sharmin S, Jakariya MD, Rahman M, Dear K, Smith W. 2004. Association between nutritional status and arsenicosis due to chronic arsenic exposure in Bangladesh. International Journal of Environmental Health Research 14:99–108.

Mitra SR, Mazumder DNG, Basu A, Block G, Haque R, Samanta S, Ghosh N, Smith MMH, von Ehrestein OS, Smith AH. 2004. Nutritional factors and susceptibility to arsenic-caused skin lesions in West Bengal, India. Environmental Health Perspectives 112:1104–1109.

Miyazaki K, Watanabe C, Mori K, Yoshida K, Ohtsuka R. 2005. The effects of gestational arsenic exposure and dietary selenium deficiency on selenium and selenoenzymes in maternal and fetal tissues in mice. Toxicology 208: 357–365.

Mo J, Zia Y, Wade TJ, Schmitt M, Le XC, Dang R, Mumford. 2006. Chronic arsenic exposure and oxidative stress: OGG1 expression and arsenic exposure, nail selenium, and skin hyperkeratosis in Inner Mongolia. Environmental Health Perspectives 114:835–841.

NRC. 1999. National Research Council's Subcommittee on Arsenic in Drinking Water. Arsenic in Drinking Water. Washington, DC: National Academy Press. p. 177–192.

Nriagu JO, Lawson G, Wong HKT, Aczue JM. 1993. A protocol for minimizing contamination in the analysis of trace metals in Great Lakes waters. Journal of Great Lakes Research 19:175–182.

Platz EA, Helzlsouer KJ, Hoffman SC, Morris JS, Baskett CK, Comstock GW. 2002. Prediognostic toenail cadmium and zinc and subsequent prostate cancer risk. The Prostate 52:288–296.

Ramanathan K, Anusuyadevi M, Shila S, Panneerselvam C. 2005. Ascorbic acid and α-tocopherol as potent modulators of apoptosis on arsenic induced toxicity in rats. Toxicology Letters 156:297–306.

Rogers MA, Thomas DB, Davis S, Weiss NS, Vaughan TL, Nevissi AE. 1991. A case-control study of oral cancer and pre-diagnostic concentrations of selenium and zinc in nail tissue. International Journal of Cancer 48:182–188.

Salbe AD, Morris VC, Levander OA. 1993. Selenium content of rat hair, nails and other tissues as affected by concurrent exposure to toxic elements. Nutrition Research 12:31–36.

Samanta G, Chowdhury TR, Mandal BK, Biswas BK, Chowdhury UK, Basu GK, Chanda CR, Lodh D, Chakraborti D. 1999. Flow injection hydride generation atomic absorption spectrometry for determination of arsenic in water and biological samples from arsenic-affected districts of West Bengal, India, and Bangladesh. Microchemistry Journal 62:174–191.

Schmitt MT, Schreinemachers D, Wu K, Ning Z, Zhao B, Le XC, Mumford JL. 2005. Human nails as a biomarker of arsenic exposure from well water in Inner Mongolia: comparing atomic fluorescence spectrometry and neutron activation analysis. Biomarkers 10:95–104.

Schulte PA. 2001. Validation of biomarkers for environmental health research and risk assessment. In: Biomarkers in Risk Assessment: Validity and Validation. Environmental Health Criteria 222. Geneva: World Health Organization. p. 202–234.

Shaaban SY, El-Hodhod MAA, Nassar MF, Hegazy AE, El-Arab SE, Shaheen FM. 2005. Zinc status of lactating Egyptian mothers and their infants: effect of maternal zinc supplementation. Nutrition Research 25:45–53.

Slotnick MJ, Nriagu JO. 2006a. Validity of human nails as a biomarker of arsenic and selenium exposure: a review. Environment Research 102:125–139.

Slotnick MJ, Nriagu JO. 2006b. Metal contamination of dietary supplements. In: Mineral Components in Foods. Szefer P, Nriagu JO, editors. New York: CRC Press. p. 433–446.

Slotnick MJ, Meliker JR, AvRuskin GA, Ghosh D, Nriagu JO. 2007. Toenails as a biomarker of inorganic arsenic intake from drinking water and foods. Journal of Toxicology & Environmental Health 70:148–158.

Slotnick MJ, Meliker JR, Nriagu JO. 2006. Effect of time and point-of-use devices on arsenic levels in southeastern Michigan drinking water. The Science of the Total Environment 369:42–50.

Slotnick MJ, Nriagu JO, Johnson M, Linder AM, Savoie KL, Jamil HJ, Hammad AS. 2005. Profiles of trace elements in toenails of Arab Americans in the Detroit area, Michigan. Biological Trace Element Research 107:113–126.

Steinmaus C, Yuan Y, Bates MN, Smith AH. 2003. Case-control study of bladder cancer and drinking water arsenic in the western United States. American Journal of Epidemiology 158:1193–1201.

Steinmaus C, Carrigan K, Kalma D, Atallah R, Yuan Y, Smith AH. 2005. Dietary intake and arsenic methylation in a U.S. population. Environmental Health Perspectives 113:1153–1159.

Takagi Y, Matsuda S, Imai S, Ohmori Y, Masuda T, Vinson JA, Mehra MC, Puri BK, Kaniewski A. 1988. Survey of trace elements in human nails: an international comparison. Bulletin of Environmental Contamination & Toxicology 41:690–695.

Vance DE, Ehmann WD, Markesbery WR. 1988. Trace element content in fingernails and hair of nonindustrialized US control population. Biological Trace Element Research 17:109–121.

Vahter M, Marafante E. 1987. Effects of low dietary intake of methionine, choline or proteins on the biotransformation of arsenite in the rabbit. Toxicology Letters 37:41–46.

Verret WJ, Chen Y, Ahmed A, Islam T, Parvez F, Kibriya MG, Graziano JH, Ahsan H. 2005. A randomized, double-blind placebo-controlled trial evaluating the effects of vitamin E and selenium on arsenic-induced skin lesions in Bangladesh. Journal of Occupational & Environmental Medicine 47:1026–1035.

Vinceti M, Bassissi S, Malagoli C, Pellacani G, Alber D, Bergomi M, Seidenari S. 2005. Environmental exposure to trace elements and risk of cutaneous melanoma. Journal of Exposure Analysis & Environmental Epidemiology 15:458–462.

Wilhelm M, Hafner D, Lombeck I, Ohnesorge FK. 1991. Monitoring of cadmium, copper, lead and zinc status in young children using toenails: comparison with scalp hair. The Science of the Total Environment 103:199–207.

Yang L, Wang W, Hou S, Peterson PJ, Williams WP. 2002. Effects of selenium supplementation on arsenism: an intervention trial in inner Mongolia. Environmental Geochemistry & Health 24:359–374.

Yetley EA. 2007. Multivitamin and multimineral dietary supplements: definitions, characterization, bioavailability, and drug interactions. American Journal of Clinical Nutrition 85 (Suppl.):269S–276S.

Zeng H. 2001. Arsenic suppresses necrosis induced by selenite in human leukemia HL-60 cells. Biological Trace Element Research 83:1-15.

Zeng H, Uthus EO, Combs GF. 2005. Mechanistic aspects of the interaction between selenium and arsenic. Journal of Inorganic Biochemistry 99:1269–1274.