Inorganic arsenic occurring in various forms in the environment has been classified as a definite human carcinogen (group 1) since 1979 (International Agency for Research on Cancer 1980). Numerous studies conducted in Taiwan and South America found that exposure to inorganic arsenic from drinking water is associated with cancers of the bladder, kidneys, skin, and other organs and tissues (Cantor 1997; Ferreccio et al. 2000).

Similar to Taiwan, several countries in South Asia have high levels of naturally occurring arsenic in groundwater. In the early 1970s, the government of Bangladesh, with the support and financing of the United Nations Children’s Fund, promoted the digging of the tube wells to provide clean drinking water. In the late 1990s, evidence indicated that the groundwater, the main source of drinking water in Bangladesh, is contaminated by naturally occurring arsenic in 59 of the 64 districts of the country. An estimated 25–40 million of Bangladesh’s 127 million people have been exposed to levels frequently reaching as high as 800 ppb (British Geological Survey 2006).

Several studies have shown convincing evidence of the association between drinking arsenic-rich water and skin lesions, which are recognized as precursors of nonmelanoma skin cancer (Ahsan et al. 2006b; Guha Mazumder et al. 2001; Tondel et al. 1999). Recent studies conducted in South Asia have raised the possibility that antioxidants may modify the effects of water arsenic on the risk of skin lesions (Hsueh et al. 1995; Vahter 2000). Folate and cobalamin (vitamin B12) have been suggested to play an important role in the detoxification of ingested arsenic (Gamble et al. 2005b; Mitra et al. 2004; National Research Council 1999). Specifically, methylation of arsenic is a cobalamin-dependent reaction catalyzed by a folate-dependent enzyme (Gamble et al. 2005b; Zakharyan and Aposhian 1999). Steinmaus et al. (2005) showed that consumption of high levels of niacin (vitamin B3) was associated with arsenic methylation. Other studies showed some evidence that antioxidants such as vitamin A also play a role in diminishing arsenic toxicity (Chattopadhyay et al. 2002; Hsueh et al. 1995; Roychowdhury et al. 2003; Styblo and Thomas 2001). To date, few studies have evaluated the effects of individual-level water arsenic measurements. We recently reported an increased dose-related risk of skin lesions in relation to arsenic exposure in this cohort (Ahsan et al. 2006b). As part of the investigation, we collected detailed information about the daily diet of all participants, using a food frequency questionnaire (FFQ) developed specifically for this population. The U.S. Department of Agriculture (USDA 2006) and Indian nutritional tables (Gopalan et al. 1996) were used to estimate the consumption of various vitamins and antioxidants. Here, using the baseline data of the HEALS cohort, we report the results of analyses aimed at clarifying the effects of the vitamin B group, including thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pyridoxine (vitamin B6), cobalamin (vitamin B12), and folic acid and antioxidants (vitamins A, C, and E) on the relationship between arsenic exposure and skin lesions.

Materials and Methods

The detailed description of the study methods and participants of the HEALS cohort analyzed here has been published previously (Ahsan et al. 2006a, 2006b; Parvez et al. 2006). In brief, we identified and tested all 5,996 tube wells in the 25-km2 area of Araihazar, Bangladesh, and proceeded to recruit eligible cohort members from their 65,876 users. A total of 14,828 individuals met the following eligibility criteria: married and ≥ 18 years of age; resident in the study with intake of fruits and canned goods, but were not able to assess the effects of individual food compounds.

The Health Effects of Arsenic Longitudinal Study (HEALS) was established to examine the health effects of arsenic exposure in Bangladesh. It is a population-based prospective cohort study in Araihazar, Bangladesh, with individual-level water arsenic measurements. The purpose of this study was to clarify the effects of the vitamin B group (thiamin, riboflavin, niacin, pyridoxine, and cobalamin) and antioxidants (vitamins A, C, and E) on arsenic-related skin lesions.

METHODS: We performed a cross-sectional study using baseline data from the Health Effects of Arsenic Longitudinal Study (HEALS), 2000–2002, with individual-level, time-weighted measures of arsenic exposure from drinking water. A total of 14,828 individuals meeting a set of eligibility criteria were identified among 65,876 users of all 5,996 tube wells in the 25-km2 area of Araihazar, Bangladesh; 11,746 were recruited into the study. This analysis is based on 10,628 subjects (90.5%) with nonmissing dietary data. Skin lesions were identified according to a structured clinical protocol during screening and confirmed with further clinical review.

RESULTS: Riboflavin, pyridoxine, folic acid, and vitamins A, C, and E significantly modified risk of arsenic-related skin lesions. The deleterious effect of ingested arsenic, at a given exposure level, was significantly reduced (ranging from 46% reduction for pyridoxine to 68% for vitamin C) for persons in the highest quintiles of vitamin intake.

CONCLUSIONS: Intakes of B-vitamins and antioxidants, at doses greater than the current recommended daily amounts for the country, may reduce the risk of arsenic-related skin lesions in Bangladesh.

Key words: antioxidants, arsenic, Bangladesh, B vitamins, skin lesions. Environ Health Perspect 116:1056–1062 (2008). doi:10.1289/ehp.10707 available via http://dx.doi.org/ [Online 16 April 2008]
area for at least 5 years before recruitment; and user of one of the study wells for at least 3 years. Nineteen percent of the eligible individuals \( n = 2,778 \) were not at home during study visits. Of the 12,050 individuals who were available and approached, 11,746 (97.5% response rate) were recruited to the cohort between October 2000 and May 2002. Informed consent was obtained from each eligible respondent who agreed to participate in the study. The study protocol and field procedures were approved by the Columbia University Institutional Review Board and by the Ethical Committee of the Bangladesh Medical Research Council.

**Measurement of arsenic exposure.** Water samples from all tube wells were analyzed for arsenic concentrations by the graphite furnace arsenic absorption method, as described previously (van Geen et al. 2003). Detailed information about water consumption in the preceding years from questionnaires was used to construct the individual-level, time-weighted arsenic (TWA) exposure measure, taking into account both arsenic concentration and duration of water drinking from the index tube well (Ahsan et al. 2006a). Thus, TWA (in micrograms per liter) = \( \Sigma C_i / T \Sigma T_i \), where \( C_i \) and \( T_i \) denote the well arsenic concentration and drinking duration for the \( i \)th well, respectively.

All HEALS participants provided urine samples, which were used to estimate total urinary arsenic concentration by the graphite furnace arsenic absorption method, as described previously (Nixon et al. 1991). Urinary creatinine was analyzed using a method based on the Jaffe reaction (Slot 1965).

**Measurement of vitamins and antioxidants.** The baseline interview collected information on diet using a semiquantitative 39-item FFQ designed to assess the long-term usual diet of cohort participants and described in detail elsewhere (Chen et al. 2004). Briefly, HEALS investigators, with help from local nutrition experts, first identified all the food items available at the village market in the study area. The FFQ was finalized after pilot testing to include common food items. Food items with intake frequencies less than once per month during the past year were deemed to be insignificant. Food diversity is limited by the low availability of food at the village market in the study area. The principle is based on dividing the entire body skin surface into 11 segments (e.g., front of arm, back of arm, face) and assigning percentages to each of them based on their size relative to the whole body surface. Each subject was examined by a physician of the same sex, who recorded presence or absence of skin lesions and their size and shape. A total of 810 cases with skin lesions (hyperkeratosis and/or melanosis) were identified among 11,746 subjects; 714 (88.1%) of those with skin lesions were confirmed upon further clinical review (421 [337 males and 84 females] had melanosis only, and 293 [247 males and 46 females] had both hyperkeratosis and melanosis (Ahsan et al. 2006b).

When we evaluated the dose-dependent effect of arsenic separately for early-stage (melanosis) and late-stage (hyperkeratosis) skin lesions, the results were similar (Ahsan et al. 2006b). In the current analysis, the modifying effects of individual B vitamins and antioxidants did not differ by severity of skin lesions (data not shown), and therefore all subsequent analyses are presented for all skin lesions.

**Statistical analysis.** Our first approach was to compare prevalence of skin lesions in the baseline cohort across the arsenic dose range. Others have suggested that the effects of many risk factors may vary among subgroups, depending on intake of dietary factors (Rothman and Greenland 1998; Willett 1998). Thus, our second statistical approach was to conduct categorical analysis of the data using unconditional logistic regression modeling for Bernoulli data (Breslow and Day 1987). In the model with water arsenic consumption, we estimated prevalence odds ratios (PORs) of skin lesions in quintiles of intake of various micronutrients. The cutoff points for these quintiles were chosen to evenly distribute all subjects. Given reasonable assumptions about the progression of such lesions to a clinically detectable state, the POR is a good approximation of the relative risk of skin lesions estimated from studies that do not involve screening (Ahsan et al. 2006b).

Most studies to date have concentrated on evaluating whether the relative risk associated with the main exposure of interest is constant across categories of the third variable, that is, under the assumption of multiplicative interaction (Rothman and Greenland 1998). We used an excess relative risk (ERR) model to estimate the ERR per unit of arsenic exposure. By adding 1.0 to the ERR, we obtained the relative risk per unit change in exposure. This risk model has the form

\[
\gamma(z_0)(1 + \delta(\text{dose}, z_0, \text{err}) \cdot t(z_0)), \tag{1}
\]

where \( \gamma, \delta, \) and \( t \) are functions of the background risks, the dose-related risks, and the risk-modifying factors, respectively; \( z_0 \) is a possible independent factor; \( z_0 \) is a risk-modifying factor; and \( \beta_{\text{dose}} \) is an arsenic dose parameter (ERR) to be estimated from the linear function of dose. Adjusted parameter estimates from this model can be directly (i.e., without exponentiation) interpreted as the increase in risk of skin lesions per unit dose of exposure in this population. Thus, any risk associated with arsenic exposure multiplies the background risk from independent risk factors other than arsenic, such as age and education, and the relationship between risk and arsenic dose is linear. Furthermore, the model gives effect estimates for the main effects of arsenic exposure, other independent risk factors, and...
potential modifying risk factors. The same model can be used to test effect modification on multiplicative scale efficiently (with fewer degrees of freedom), compared with methods based on conventional stratified analysis and the use of cross-product terms in unconditional logistic regression models.

Based on the literature review and previous analysis of the baseline data (Ahsan et al. 2006b; Argos et al. 2007), we specified a number of a priori confounders for the arsenic–skin lesion relationship such as sex, age (categorized into nine 5-year categories), body mass index (BMI; categorized into quintiles of an approximately equal number of subjects), education (< 1, 1–6, 6–11, and > 11 years), occupation, and television and land ownership (proxies of socioeconomic status). Several studies (e.g., Chen et al. 2006; McCarty et al. 2006), including our previous analysis of this cohort (Ahsan et al. 2006b), have shown that the use of tobacco products is associated with the risk of skin lesions independently of arsenic exposure.

We evaluated effects of consumption of various B vitamins (thiamin, riboflavin, niacin, pyridoxine, cobalamin, and folic acid) and antioxidants (vitamins A, C, and E) on arsenic-related skin lesions by two methods: we evaluated them for independent associations with skin lesions, and we used Equation 1 to evaluate their effect-modifying properties. When variables were evaluated as possible independent risk factors of skin lesions, an arsenic dose parameter was retained in the model to control for the main effects of dose and for possible confounding effects. Individual risk factors were retained in the model if they significantly improved the fit of the model, as evaluated by the likelihood ratio test comparing the deviances from the two nested models or if the likelihood ratio test comparing the nested model with a model with additional variable.

The use of cross-product terms in unconditional multiplicative scale efficiently (with fewer potential modifying risk factors. The same model can be used to test effect modification on multiplicative scale efficiently (with fewer degrees of freedom), compared with methods based on conventional stratified analysis and the use of cross-product terms in unconditional logistic regression models.

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In our cohort, intake of riboflavin strongly correlated with the intake of pyridoxine and folic acid (Pearson’s correlation coefficients 0.75 and 0.83, respectively; data not shown). When we investigated the combined independent effects of these three micronutrients by simultaneously entering them into the model, we observed slight attenuation of effect of arsenic-related skin lesions, but the reduction in risk in the upper quintiles of micronutrient consumption compared with the lowest quintile remained unchanged (data not shown).

Table 3 shows that after adjusting the background rates for sex, age at risk, education, BMI, and total energy consumption, those consuming water containing 131 µg/L time-weighted water arsenic had an ERR of skin lesions of 1.6 (95% CI, 1.0–2.1); that is, for every 131-µg/L increase of time-weighted water arsenic, we observed a 2.6-fold higher risk of skin lesions. The effects of arsenic exposure were modified by consumption of vitamins. Table 3 shows that intake of riboflavin (model 2) was a strong modifier of the effects of arsenic. Those in the highest quintile of consumption had only 37% of the ERR (1.0 per 131 µg/L; 95% CI, 0.5–1.8) of those in the lowest quintile (ERR = 2.7 per 131 µg/L; 95% CI, 0.2–3.8). Similarly, intakes of folic acid (model 4) and vitamins A, C, and E (models 6–8) modified the arsenic-related skin lesion risks, with reductions of ERR estimates for skin lesions in the highest quintiles (compared with the lowest quintiles of micronutrient intake) ranging from 65 to 68%.

To estimate the combined effect of B vitamins on arsenic-related skin lesions, we repeated our analyses with simultaneous interaction terms for riboflavin, pyridoxine, and folic acid. We observed that those in the highest quintiles of consumption of all three micronutrients had only 37.8% of the ERR of those in the lowest quintiles of consumption for all three nutrients (ERR, 3.2 per 131 µg/L weighted water arsenic exposure) (model 5). Thus, on the basis of these results and those presented in Tables 2 and 3, it appears that the effects of the B vitamins are additive in nature.

We further investigated the effects of urinary arsenic, which was strongly correlated with the time-weighted arsenic exposure measure used in Table 3 (Pearson’s r = 0.47; data not shown). Similar to the analysis of the time-weighted arsenic exposure measure presented in Table 2, only riboflavin; pyridoxine; vitamins A, C, and E; and folic acid displayed an

### Table 2. PORs and 95% CIs for the effects of B vitamins and antioxidants on risk of skin lesions.

| Vitamin, energy-adjusted values (quintiles) | Adjusted POR | 95% CI        | p-Value |
|-------------------------------------------|--------------|---------------|---------|
| Thiamin (mg/day)                          |              |               |         |
| < 1.62                                    | 1            | 0.7–1.7       | 0.162   |
| 1.62–1.91                                 | 1            | 0.4–1.2       |         |
| 1.92–2.19                                 | 0.6          | 0.3–1.2       |         |
| ≥ 2.51                                    | 0.5          | 0.2–1.2       |         |
| Riboflavin (mg/day)                       |              |               |         |
| < 0.69                                    | 1            | < 0.001       |         |
| 0.69–0.82                                 | 0.7          | 0.6–1.0       |         |
| 0.83–0.96                                 | 0.6          | 0.4–0.8       |         |
| 0.97–1.16                                 | 0.6          | 0.4–0.8       |         |
| ≥ 1.17                                    | 0.5          | 0.3–0.7       |         |
| Niacin (mg/day)                           |              |               |         |
| < 23.48                                   | 1            | 0.365         |         |
| 23.48–28.67                               | 1.1          | 0.6–2.0       |         |
| 28.68–32.44                               | 1.0          | 0.5–2.1       |         |
| 32.45–37.97                               | 1.5          | 0.3–3.6       |         |
| ≥ 37.99                                   | 1.0          | 0.3–2.6       |         |
| Pyridoxine (mg/day)                       |              |               |         |
| < 2.72                                    | 1            | 0.001         |         |
| 2.72–3.17                                 | 0.8          | 0.5–1.1       |         |
| 3.18–3.65                                 | 0.5          | 0.3–0.8       |         |
| 3.65–4.18                                 | 0.3          | 0.2–0.6       |         |
| ≥ 4.19                                    | 0.4          | 0.2–0.8       |         |
| Cobalamin (mg/day)                        |              |               |         |
| < 0.84                                    | 1            | 0.200         |         |
| 0.84–1.30                                 | 0.8          | 0.6–1.1       |         |
| 1.31–1.80                                 | 1.0          | 0.8–1.3       |         |
| 1.81–2.53                                 | 0.8          | 0.6–1.0       |         |
| ≥ 2.54                                    | 0.9          | 0.6–1.1       |         |
| Folic acid (µg/day)                       |              |               |         |
| < 196.64                                  | 1            | < 0.001       |         |
| 196.64–240.63                             | 0.6          | 0.4–0.8       |         |
| 240.64–283.99                             | 0.5          | 0.4–0.7       |         |
| 284.00–351.60                             | 0.5          | 0.3–0.7       |         |
| ≥ 351.61                                  | 0.5          | 0.3–0.6       |         |
| Vitamin A (mg/day)                        |              |               |         |
| < 2298.48                                 | 1            | < 0.001       |         |
| 2298.48–3503.45                           | 0.7          | 0.5–0.8       |         |
| 3503.48–4886.75                           | 0.7          | 0.5–1.0       |         |
| 4886.76–7113.05                           | 0.7          | 0.5–0.9       |         |
| ≥ 7113.06                                 | 0.5          | 0.4–0.7       |         |
| Vitamin C (mg/day)                        |              |               |         |
| < 52.57                                   | 1            | < 0.001       |         |
| 52.57–71.82                               | 0.7          | 0.5–0.9       |         |
| 71.83–92.67                               | 0.5          | 0.3–0.6       |         |
| 92.67–128.16                              | 0.4          | 0.3–0.6       |         |
| ≥ 128.17                                  | 0.4          | 0.3–0.6       |         |
| Vitamin E (mg/day)                        |              |               |         |
| < 4.06                                    | 1            | < 0.001       |         |
| 4.06–4.79                                 | 0.5          | 0.3–0.7       |         |
| 4.80–5.52                                 | 0.5          | 0.4–0.7       |         |
| 5.53–6.40                                 | 0.4          | 0.3–0.6       |         |
| ≥ 6.41                                    | 0.4          | 0.3–0.6       |         |

*Adjusted for time-weighted water arsenic, sex, age at risk, BMI, education, and total energy intake. †Test of homogeneity of PORs across quintiles of micronutrient intake.

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**Figure 1. PORs for quintiles of vitamin intake from the categorical analysis.**

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The inverse effect on the risk of skin lesions (data not shown). Table 4 shows that, on average, ERIs associated with the urinary arsenic concentrations were > 3-fold higher than those associated with the water arsenic. Furthermore, riboflavin; pyridoxine; vitamins A, E, and C; and folic acid modify the effects of urinary arsenic. These effects are in the same direction (albeit stronger) as the effects observed for the time-weighted arsenic exposure measure in Table 3, with reductions in risk of skin lesions in the upper quintiles of vitamins intake ranging from 34 to 74%.

**Discussion**

In this article we report the results of analysis of the baseline data from the prospective cohort study of the association between time-weighted water arsenic exposure and risk of skin lesions. Although skin lesions have been linked previously with exposure to arsenic-contaminated drinking water, there is a recognized lack of information on the modifying effects of local diet on this relationship (McCarty et al. 2006). To our knowledge, this is the first systematic analysis of the association between micronutrient intake and prevalence of arsenic-induced skin lesions.

We estimated an ERR of 1.6 (95% CI, 1.0–2.1) per 131-µg/L time-weighted water arsenic and an ERR of 4.91 (95% CI, 1.8–8.1) per 130-µg/L urinary arsenic concentration. Thus, those exposed to a dose equal to 50 µg/L water arsenic, the currently permissible arsenic limit in Bangladesh (and in the United States, until recently), had a 59% higher risk of skin lesions compared with those with dose zero. Even a small dose of water arsenic equal to the current water arsenic concentration limit in the United States of 10 µg/L carried a 12% increase in risk compared with those with zero doses.

We found that riboflavin; pyridoxine; vitamins A, C, and E; and folic acid were significant strong modifiers of the effects of ingested arsenic. Our results suggest that consumption of a diet rich in these vitamins and antioxidants can significantly reduce the harmful effects of water arsenic on the development of skin lesions. Those in the highest percentile of consumption had a significant reduction in risks, ranging from 46% (ERR decreased from

**Table 3.** Modification of effect of water arsenic on skin lesions by B vitamins and antioxidants.

| Model* | Variable, energy-adjusted values (quintiles) | ERR† | 95% CI | p-Value‡ |
|--------|---------------------------------------------|------|--------|----------|
| 1      | Water arsenic (µg/L)                        | 1.6  | 1.0–2.1|          |
| 2      | Riboflavin (mg/day)                         | > 0.69 | 2.7 | 0.2–3.8 | 0.010 |
|        | 0.69–0.82                                  | 2.0  | 1.3–3.1|          |
|        | 0.83–0.96                                  | 1.3  | 0.8–2.2|          |
|        | 0.97–1.16                                  | 1.4  | 0.8–2.3|          |
|        | ≥ 1.17                                      | 1.0  | 0.5–1.8|          |
| 3      | Pyridoxine (mg/day)                         | < 2.72 | 2.4 | 1.2–3.6 | 0.054 |
|        | 2.72–3.17                                   | 2.2  | 1.3–3.7|          |
|        | 3.18–3.65                                   | 1.3  | 0.7–2.5|          |
|        | 3.66–4.18                                   | 0.8  | 0.4–1.8|          |
|        | ≥ 4.19                                      | 1.0  | 0.7–2.7|          |
| 4      | Folic acid (µg/day)                         | < 196.64 | 3.2 | 1.4–4.4 | < 0.001 |
|        | 196.64–240.63                               | 1.8  | 1.2–2.8|          |
|        | 240.64–283.99                               | 1.3  | 0.8–2.1|          |
|        | 284.00–351.60                               | 1.2  | 0.7–1.9|          |
|        | ≥ 351.61                                    | 1.1  | 0.7–1.8|          |
| 5      | Combined model: riboflavin (mg)/pyridoxine (mg)/folic acid (µg) | < 0.69/2.72/196.64 | 3.2 | 1.6–4.9 | 0.005 |
|        | 0.69–0.82/2.72–3.17/196.64–240.63           | 2.2  | 0.9–3.6|          |
|        | 0.83–0.96/3.18–3.65/240.64–283.99           | 1.3  | 0.9–1.9|          |
|        | 0.97–1.16/3.66–4.18/284.00–351.60           | 0.9  | 0.6–1.3|          |
|        | ≥ 1.17/4.19/≥ 351.61                        | 1.2  | 0.5–2.0|          |
| 6      | Vitamin A (mg/day)                          | < 2298.48 | 2.6 | 1.7–3.6 | < 0.001 |
|        | 2298.48–3503.45                             | 1.2  | 0.9–2.0|          |
|        | 3503.46–4886.75                             | 1.7  | 1.1–2.6|          |
|        | 4886.76–7113.05                             | 1.7  | 1.1–2.6|          |
|        | ≥ 7113.06                                   | 0.9  | 0.5–1.5|          |
| 7      | Vitamin C (mg/day)                          | < 52.57 | 3.4 | 2.3–4.6 | < 0.001 |
|        | 52.57–71.62                                 | 2.0  | 1.4–2.9|          |
|        | 71.63–92.67                                 | 1.2  | 0.7–1.9|          |
|        | 92.68–126.16                                | 1.1  | 0.7–1.7|          |
|        | ≥ 126.17                                    | 1.1  | 0.7–1.8|          |
| 8      | Vitamin E (mg/day)                          | < 4.06 | 3.4 | 1.9–4.9 | < 0.002 |
|        | 4.06–4.79                                   | 1.3  | 0.9–2.2|          |
|        | 4.80–5.52                                   | 1.5  | 0.9–2.5|          |
|        | 5.53–6.40                                   | 1.2  | 0.6–2.1|          |
|        | ≥ 6.41                                      | 1.2  | 0.6–2.1|          |

*Models adjusted for sex, age at risk, BMI, education, and total energy intake. †ERR of arsenic-related skin lesions per 131-µg/L time-weighted water arsenic for quintiles of micronutrient intake. ‡*t* Test using ordinal variable to represent increasing quintiles of micronutrient intake.
Finally, many participants drank water from the same well (59.2% of study participants drank water from ≥3 subjects/well, 31.9% from a well with 2 study subjects/well, and the remaining 8.9% of subjects from individual wells), making well water arsenic concentration a shared characteristic. These correlated errors arising from shared wells would affect the width of the CIs, but should not affect the magnitudes or directions of the point estimates.

Methylation of arsenic, a hypothesized detoxification pathway, requires the conversion of S-denosylmethionine to S-adenosylhomocysteine and depends partly on the one-carbon metabolism in which riboflavin, pyridoxine, cobalamin, and folic acid all play a role (Selhub 2002). Previous studies have shown that intake of vitamins influences the efficiency of arsenic methylation (McCarty et al. 2006; Steinmaus et al. 2005). Our findings that riboflavin, pyridoxine, and folic acid modified the risk of skin lesions are consistent with the hypothesis that individuals with insufficient intakes of nutrients related to arsenic metabolism are more susceptible to the health effect of arsenic exposure.

These findings further suggest that riboflavin, pyridoxine, and folic acid may play a more important role in modifying arsenic toxicity.

Although folic acid is readily available in many food items, its deficiency is not uncommon, primarily because naturally occurring folates are highly susceptible to oxidative degradation, for example, during cooking (Gamble et al. 2005b). We reported previously that there is a high prevalence of folate deficiency and hyperhomocysteinemia in Araihazar, Bangladesh (Gamble et al. 2005a) and that these conditions are associated with reduced arsenic methylation (Gamble et al. 2005b).

Intracellular antioxidants such as vitamins A, C, and E decrease arsenic toxicity by reversing disturbances in lipid peroxidation, generation of nitric oxide, reactive oxygen species, and apoptosis initiated by arsenic metabolites (Chattopadhyay et al. 2002; National Research Council 1999). β-Carotene may also scavenge free-radical species (Krinsky 1989). Previous studies have shown that low serum levels of carotene modified the effect of arsenic exposure on the risk of ischemic heart disease (Hsieh et al. 1998). Several studies have found that...
prevalence of vitamin A deficiency in Bangladesh (Ahmed 1999), possibly explaining the important modifying effect of vitamin A observed in this study. Given that vitamin A consumption in the Bangladeshi diet is mostly from plant sources, the correlation between beta carotene intake and vitamin A in our study was nearly perfect (r = 0.99), explaining the similarity of modifying effects of vitamin A and beta carotene on arsenic-related skin lesions.

Overall malnutrition, defined as either a general calorie deficit or a diet adequate in calories but nutritionally poor, is an important cofactor in arsenic poisoning affecting the timing and the intensity of arsenic-related problems. Guha Mazumder et al. (1998) showed that subjects with lower BMIs had higher prevalence of arsenic skin lesions compared with subjects with similar arsenic exposures but higher BMIs. Similarly, in our previous analysis of this cohort, we showed that study subjects with lower BMIs were at increased risk of skin lesions (Ahsan et al. 2006b). Current analysis indicates that BMI has an independent effect on the development of skin lesions, and thus, subjects with lower consumption of vitamins and antioxidants had higher risk of skin lesions, indicating significant effects of individual nutrients beyond what can be explained by general calorie intake.

Findings on the modifying effect of sunlight exposure on arsenic-related skin lesions have been described elsewhere (Chen et al. 2006). Because women in Bangladesh universally wear traditional dresses that almost completely cover the skin of their trunk, sunlight exposure of female respondents was considered minimal and therefore was not assessed in the study. In men, we observed an additive effect of higher arsenic exposure and excessive sunlight exposure, such that the risk of skin lesions associated with any given level of arsenic exposure was greater in males with excessive sun exposure. In the present analysis, we empirically assessed whether sunlight exposure is a potential confounder. Adjustment for sunlight exposure did not change the effect estimate and therefore we did not include it in the model.

Conclusion
The results of this study strongly suggest that consumption of foods rich in vitamins such as riboflavin; pyridoxine; vitamins A, C, and E; and folic acid may influence the relationship between exposure to water arsenic and subsequent risk of skin lesions. Those in the highest quintiles of consumption had significant reductions in risks for skin lesions associated with arsenic exposure. The observed modifying effects were associated with consumption of nutrients at doses that are higher than the current recommended daily amounts for the country. These findings support the hypothesis that nutrients relevant to arsenic metabolism and antioxidant nutrients may modify the risk of arsenic-related skin lesions in Bangladesh. Future studies looking at that influence of micronutrients on risk of incident arsenic-related skin lesions are necessary to clarify the nature of the association. Intervention studies are also needed to determine whether dietary supplementation may mediate health effects of arsenic exposure.

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