Intakes of Several Nutrients Are Associated with Incidence of Arsenic-Related Keratotic Skin Lesions in Bangladesh¹,²

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Abstract

Risk of skin lesions due to chronic arsenic exposure can be further affected by nutrient intake. We prospectively evaluated the association of nutrient intake and gender with incident skin lesions using data from the Health Effects of Arsenic Longitudinal Study (HEALS) in Araihazar, Bangladesh. Discrete time hazard models were used to estimate these effects in stratified analyses based on skin lesion severity. Overall, we observed significant associations between low intakes of various nutrients (retinol, calcium, fiber, folate, iron, riboflavin, thiamin, and vitamins A, C, and E) and skin lesion incidence, particularly for keratotic skin lesions. Associations for vitamins C and E showed significant linear trends. Gender-specific analyses revealed an inverse association between the lowest quartile of nutrient intake and keratotic skin lesion incidence for retinol equivalents, calcium, folate, iron, and fiber among women. Interactions by gender were observed for retinol equivalents (P-interaction = 0.03), calcium (P-interaction = 0.04), vitamin A (P-interaction = 0.03), and riboflavin (P-interaction = 0.04) with the incidence of keratotic skin lesions. Understanding differential susceptibility to skin lesion incidence based on nutrient intake will help researchers develop targeted interventions to prevent health consequences of arsenic poisoning in Bangladesh and beyond. J. Nutr. 142: 2128–2134, 2012.

Introduction

Inorganic arsenic is a class 1 human carcinogen that is readily absorbed into the bloodstream via drinking water and has been linked to a variety of adverse health outcomes, including cancers of the skin, lung, bladder, liver, and kidney (1–3). Arsenic-associated cancers are considered a late phenomenon related to long-term exposure to arsenic, usually developing after at least 10 y of exposure (4). Skin lesions, however, can appear within a few years of exposure and usually progress through stages. Typically, the progression begins with the hyper-pigmentation of the skin in a “raindrop” pattern known as melanosis. This is often accompanied by hypo-pigmented areas, presenting as whitish, rounded dotts giving rise to a pattern referred to as leucomelanosis. In addition to changes in pigmentation, hyperkeratosis, generally the most severe form, is defined as the bilateral thickening of the palms and soles. Skin lesions may progress to nonmelanoma skin cancers and are therefore a good intermediate endpoint or predictive indicator to investigating arsenic-related carcinogenesis (5).

The Health Effects of Arsenic Longitudinal Study (HEALS) was initiated in 2000 to prospectively investigate the relationship between chronic arsenic exposure and adverse health outcomes in a population chronically exposed to arsenic via contaminated drinking water. Given that malnutrition is common in Bangladesh, studies of diet and arsenic exposure are necessary to understand the potential impact that insufficient nutrition can have on skin lesion incidence. Several descriptive epidemiologic investigations suggest that individuals with diets deficient in nutrients from vegetables and animal products may experience higher arsenic toxicity due to less efficient arsenic metabolism and that metabolism efficiency may also be affected by factors such as gender (6–8). Previous findings suggest that greater intakes of methionine, cysteine, and protein and vitamins such as thiamin and niacin resulted in greater urinary total arsenic excretion (6,9). The intake of B vitamins and folate has also been associated with reduced skin lesion prevalence (10). Finally, diets rich in gourd and root vegetables may reduce arsenical skin lesion risk in this population (11). In this study, we utilized food frequency derived-nutrient intake in the HEALS population to evaluate associations of various nutrients with skin lesion incidence, adjusting for arsenic exposure and other potentially confounding factors. It has been suggested that men and women living in South Asian countries like Bangladesh have...
Materials and Methods

Study area and study population. The HEALS is a prospective investigation of health outcomes associated with chronic arsenic exposure through drinking water in a population-based sample of adults in Araihazar, Bangladesh (13,14). Detailed information on study methodology and sampling was previously described. In short, a population-based sampling frame was created by collecting water samples, geographic data, and basic demographic information on the users of 5966 contiguous wells in a 25-km² study area (15). Beginning in October 2000, 11,746 married individuals aged 18–75 y were recruited for inclusion in the cohort. Subsequently, a roster was established based on well water arsenic measurements from a separate set of 5000 wells and an additional 8287 individuals were recruited in 2007 using the same study methodologies. The participants were subsequently visited every 2 y for follow-up evaluation, which consisted of an interviewer-administered questionnaire, physician-administered clinical examination, and urine sample collection. All participants underwent a verbal consent procedure after the study objectives and procedures were explained. The study procedures were approved by the University of Chicago and Columbia University Institutional Review Boards and the Ethical Committee of the Bangladesh Medical Research Council. At baseline, trained research physicians, unaware of the arsenic level of the participants’ drinking water, conducted in-person interviews that included questions concerning water drinking patterns and history as well as demographic information and lifestyle characteristics.

In addition to the interview data, urine and blood samples were collected from participants in their homes according to a structured protocol. At recruitment, whole venous blood samples were collected in a 10-mL vacutainer tube containing serum separator and in a 2-mL EDTA tube. Additionally, spot urine samples were collected in 50-mL acid-washed tubes. Urine and blood samples were stored in coolers until transfer to −20°C freezers in the study office until analysis (15). A comprehensive physical examination was also conducted with a specific focus on the signs and symptoms of arsenic-related diseases, including skin lesions.

Nutrient intake assessment. Dietary intakes were assessed at baseline using a previously validated, semiquantitative, 39-item FFQ designed for the HEALS (11). Focus groups were initially conducted to determine the food items to include in the questionnaire. Validity was determined using a 7-d food diary from 189 randomly selected participants (16). Correlations between the food group intakes ascertained from the FFQs and the 7-d food diaries ranged from 0.19 to 0.78. Total energy, macronutrient, and micronutrient intakes were estimated utilizing the USDA Nutrient Database for Standard Reference (17). These values were also estimated utilizing a database of foods derived from the RDA for India (16). All nutrient intakes were adjusted for total energy intake by using the residual method (11,16). Each of the food items was categorized into quintiles based on the distribution of the nutrients within the study sample.

Skin lesion status. At baseline and each follow-up interview, skin lesions were ascertained using a structured protocol by trained study physicians (11,15). Through the whole-body examination, the study physician recorded the presence or absence of melanosis, leucolena-

sis, and keratosis as well as their location, size, and shape. For the present analysis, skin lesion status was classified as either keratotic (with or without melanosis and/or leucolena) or nonkeratotic (melanosis and/or leucolena only). For the purposes of these analyses, once an individual was censored, there was no reentry into the analysis cohort.

Covariate assessment. The well water arsenic concentrations of all of the tube wells assessed in the study were measured using previously described, laboratory-based methods (13,14). In short, the water arsenic concentrations were measured using graphite furnace atomic absorption spectrometry with a detection limit of 5 μg/L. Samples below the limit of detection were subsequently reassayed by inductively coupled plasma MS with a detection limit of 0.1 μg/L (13). At baseline, participants were asked to identify the well used as their primary source of drinking water. Corresponding well water arsenic exposure was subsequently assigned based on this response.

All covariate data were derived from the baseline interview. Sociodemographic and lifestyle factors included age (continuous years), smoking status (never, current, former), quintiles of water arsenic intake, years of education (continuous), and formal education (yes, no). BMI [weight (kg)/height² (m²)] was derived from measured height and weight at baseline. Standard international cutoff points were used to classify participants into underweight (<18.5), healthy weight (18.5–24.9), and overweight (≥25.0) categories. Well water arsenic cutoffs for the first and second well water quintiles at baseline were adjusted to correspond to the WHO’s guideline for arsenic in drinking water (≤10 μg/L) and the national standard for arsenic in drinking water in Bangladesh (≤50 μg/L), respectively (4,15).

Exclusions and eligibility. Individuals with prevalent skin lesions at baseline (n = 1162) and individuals who did not receive skin examinations at baseline or at subsequent follow-ups (n = 870) were excluded from the present analysis, resulting in a cohort of 17,971 individuals. Additionally, we excluded individuals with incomplete FFQ data (n = 412) or implausible total energy intakes (n = 1168; <500 and >3500 kcal/d for females and >4000 kcal/d for males), resulting in a total of 16,391 individuals.

Statistical analysis. Discrete time hazard models were used to estimate discrete time HRs and their 95% CIs for skin lesions. These models are based on the probability, or the discrete time hazard, of skin lesion incidence at each study interval conditional on being skin lesion-free at the previous study interval (18). The conditional probability of a new lesion was estimated using a log-linear model with common regression coefficients across all intervals but with different intercepts for each interval. Regression coefficients are log discrete time HRs, analogous to continuous time HRs corresponding to traditional proportional hazards models (19). We employed robust SEs for discrete time hazards to account for potential correlation due to the clustered sampling of individuals within each primary well (20,21).

Nutrient quartiles were modeled using dummy categories, wherein the reference category was always chosen to be the highest nutrient consumption group for each nutrient. A single ordinal variable was included in the regression model and the corresponding Wald chi-squared statistic was used as the test for trend across categories of nutrient intakes. Analyses were also conducted with nutrient intakes based on RDAs for India and yielded similar results (data not shown). BMI, years of education, water arsenic quintiles, smoking status, gender, and follow-up interval were included in the analysis as potential confounders based on our a priori understanding of causal factors related to arsenic-related skin lesions. Stratified analyses were conducted based on the severity of skin lesions and tested the association of nutrient intake with keratotic and nonkeratotic skin lesions separately.

Additionally, potential effect modifications by gender were evaluated for the associations between nutrients and skin lesions. This was implemented by including a cross-product term of the ordinal nutrient intake variable and the binary gender variable as an ordinal term in the fully adjusted, stratified model for a given skin lesion outcome. These models test whether the linear test for trend for a nutrient is statistically different between men and women. Statistical analyses were performed using SAS, including the procedure GENMOD for analysis release 9.2 (SAS Institute) and STATA version 11 (Stata Corporation).

Results

Among the 16,391 individuals available for analysis, 885 incident skin lesions were detected during the follow-up period.
We observed an inverse association between nutrient intake and incidence of keratotic skin lesions in women, with larger effects for the lowest intake quartile of retinol equivalents [HR = 2.56 (95%CI: 1.14–5.74)], thiamin [HR = 2.56 (95%CI: 1.07–6.12)], vitamin E [HR = 2.75 (95%CI: 1.30–5.83)], vitamin C [HR = 2.30 (95%CI: 1.08–4.86)], and riboflavin [HR = 2.55 (95%CI: 1.06–6.16)].

Trends for the associations between nutrient intake and incidence of keratotic skin lesions were observed in women, including fiber (P-trend = 0.03), folate (P-trend = 0.03), riboflavin (P-trend = 0.03), and vitamin E (P-trend = 0.02). For each of these nutrient categories, particularly the lowest intake quartile, decreased intake significantly increased the risk of keratotic skin lesions in women. Marginally significant trends were observed for retinol equivalents (P-trend = 0.05) and calcium (P-trend = 0.05).

Significant multiplicative interactions between gender and nutrient intakes for keratotic skin lesion risk were observed for retinol equivalents (P-interaction = 0.03), calcium (P-interaction = 0.03), riboflavin (P-interaction = 0.04), and vitamin A (P-interaction = 0.03).

### Discussion

In these longitudinal analyses based on data from the HEALS cohort, we evaluated the associations between nutrient intake and incidence of keratotic skin lesions in women. Marginally significant trends were observed for retinol equivalents (P-trend = 0.05) and calcium (P-trend = 0.05).

Significant multiplicative interactions between gender and nutrient intakes for keratotic skin lesion risk were observed for retinol equivalents (P-interaction = 0.03), calcium (P-interaction = 0.03), riboflavin (P-interaction = 0.04), and vitamin A (P-interaction = 0.03).

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No skin lesion (n = 15,506)</th>
<th>Nonkeratotic skin lesion (n = 726)</th>
<th>Unadjusted</th>
<th>Keratotic skin lesion (n = 159)</th>
<th>Unadjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>HR (95% CI)</td>
<td>n (%)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Water arsenic, µg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1–10</td>
<td>4945 (32.1)</td>
<td>135 (18.6)</td>
<td>1.00</td>
<td>21 (13.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>10.1–50</td>
<td>3641 (23.7)</td>
<td>118 (16.2)</td>
<td>1.11 (0.86–1.44)</td>
<td>24 (15.1)</td>
<td>1.36 (0.76–2.45)</td>
</tr>
<tr>
<td>50.1–100</td>
<td>2743 (17.8)</td>
<td>125 (17.2)</td>
<td>1.56 (1.21–2.00)</td>
<td>28 (17.6)</td>
<td>2.11 (1.19–3.73)</td>
</tr>
<tr>
<td>100.1–200</td>
<td>2421 (15.7)</td>
<td>173 (23.8)</td>
<td>1.92 (1.51–2.43)</td>
<td>32 (20.1)</td>
<td>2.04 (1.17–3.73)</td>
</tr>
<tr>
<td>≥200.1</td>
<td>1616 (10.5)</td>
<td>176 (24.2)</td>
<td>2.76 (2.17–3.52)</td>
<td>54 (34.0)</td>
<td>4.71 (2.85–7.78)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10,011 (64.5)</td>
<td>212 (29.2)</td>
<td>1.00</td>
<td>48 (30.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Male</td>
<td>5495 (35.4)</td>
<td>515 (70.8)</td>
<td>4.18 (3.56–4.90)</td>
<td>111 (69.8)</td>
<td>3.99 (2.86–5.55)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–30</td>
<td>5603 (36.1)</td>
<td>68 (9.40)</td>
<td>1.00</td>
<td>12 (7.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>31–40</td>
<td>5017 (32.3)</td>
<td>201 (27.7)</td>
<td>2.79 (2.12–3.68)</td>
<td>36 (23.9)</td>
<td>2.92 (1.52–5.60)</td>
</tr>
<tr>
<td>41–50</td>
<td>3415 (22.0)</td>
<td>250 (34.4)</td>
<td>5.70 (4.34–7.48)</td>
<td>70 (44.0)</td>
<td>9.12 (5.00–16.6)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>6026 (39.0)</td>
<td>322 (44.7)</td>
<td>1.00</td>
<td>71 (44.9)</td>
<td>1.00</td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>8301 (53.7)</td>
<td>368 (51.0)</td>
<td>0.82 (0.71–0.95)</td>
<td>80 (50.6)</td>
<td>0.81 (0.59–1.11)</td>
</tr>
<tr>
<td>≥25</td>
<td>1123 (7.27)</td>
<td>37 (5.3)</td>
<td>0.51 (0.35–0.74)</td>
<td>7 (4.33)</td>
<td>0.52 (0.24–1.13)</td>
</tr>
<tr>
<td>Education, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6671 (43.0)</td>
<td>349 (48.0)</td>
<td>1.00</td>
<td>79 (49.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>1–5</td>
<td>4803 (30.9)</td>
<td>203 (28.0)</td>
<td>0.85 (0.71–1.00)</td>
<td>54 (34.0)</td>
<td>1.00 (0.71–1.41)</td>
</tr>
<tr>
<td>≥6</td>
<td>4024 (25.9)</td>
<td>174 (24.0)</td>
<td>0.84 (0.70–1.01)</td>
<td>26 (16.4)</td>
<td>0.56 (0.36–0.86)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>11,170 (72.1)</td>
<td>276 (38.0)</td>
<td>1.00</td>
<td>60 (37.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>Former</td>
<td>794 (5.12)</td>
<td>120 (16.5)</td>
<td>5.59 (4.55–6.88)</td>
<td>20 (12.6)</td>
<td>4.39 (2.67–7.22)</td>
</tr>
<tr>
<td>Current</td>
<td>3539 (22.8)</td>
<td>331 (45.5)</td>
<td>3.43 (2.93–4.02)</td>
<td>79 (49.7)</td>
<td>3.72 (2.69–5.15)</td>
</tr>
</tbody>
</table>

1 HR represents an unadjusted discrete time HR. HEALS, Health Effects of Arsenic Longitudinal Study.
and skin lesion incidence and whether gender modifies these associations. An important finding from the present study is that deficiency in various nutrients may increase the risk of arsenic-induced skin lesions and may affect skin lesion progression based on lesion severity. We saw significant linear trends and associations between risk of keratotic skin lesions and the lowest quartile of nutrient intakes for retinol, calcium, fiber, folate, iron, riboflavin, thiamin, and vitamins A, C, and E. Another major finding was the observation that in general, nutrient effects were more pronounced among women. The strongest evidence of this was seen for low intakes of retinol equivalents, which is derived from various dietary components (6). It has been shown in animal bioassays that dietary deficiencies of certain nutrients such as choline and folate decreased urinary arsenic excretion and increased retention of arsenic in the tissue. Additionally, a previous study suggested that high intakes of protein methionine and cysteine were associated with increased arsenic excretion and an increased urinary concentration of arsenic metabolites (6). A previous study also identified a significant association between dietary fiber intake and skin lesion incidence and whether gender modifies these associations (22). Several authors have suggested that these dietary factors play an important role in arsenic metabolism.

The process of arsenic metabolism involves the conversion of inorganic arsenic to monomethylarsonic acid and dimethylarsonic acid, so that it can be excreted from the body via urine (8,11). The biochemical pathways involved in the methylation of arsenic are dependent on the availability of S-adenosylmethionine, which is derived from various dietary components (6). It has been shown in animal bioassays that dietary deficiencies of certain nutrients such as choline and folate decreased urinary arsenic excretion and increased retention of arsenic in the tissue. Additionally, a previous study suggested that high intakes of protein methionine and cysteine were associated with increased arsenic excretion and an increased urinary concentration of arsenic metabolites (6). A previous study also identified a significant association between dietary fiber intake and skin lesion risk. Though the explanation for this association is yet to be determined, fiber potentially plays a role via the absorption of arsenic through the gastrointestinal tract (22).

In addition to dietary factors that influence arsenic metabolism, it has also been suggested that gender plays an important role in arsenic metabolism. In a separate study of a Bangladeshi population, the authors showed that women in general had higher arsenic methylation efficiency than men, primarily in

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Q4 (highest)</th>
<th>Q3</th>
<th>Q2</th>
<th>Q1 (lowest)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol equivalents, μg/d</td>
<td>4439</td>
<td>4331</td>
<td>4245</td>
<td>4246</td>
<td>2.36 (1.06–5.27)</td>
</tr>
<tr>
<td>Calcium, mg/d</td>
<td>4422</td>
<td>4265</td>
<td>4221</td>
<td>4353</td>
<td>2.56 (1.04–6.25)</td>
</tr>
<tr>
<td>Carbohydrate, g/d</td>
<td>4369</td>
<td>4113</td>
<td>4339</td>
<td>4440</td>
<td>0.66 (0.21–1.50)</td>
</tr>
<tr>
<td>Cholesterol, mg/d</td>
<td>4385</td>
<td>4387</td>
<td>4356</td>
<td>4133</td>
<td>1.36 (0.49–3.72)</td>
</tr>
<tr>
<td>Copper, mg/d</td>
<td>3860</td>
<td>4285</td>
<td>4504</td>
<td>4612</td>
<td>1.87 (0.84–3.84)</td>
</tr>
<tr>
<td>Monounsaturated fats, g/d</td>
<td>4432</td>
<td>4197</td>
<td>4182</td>
<td>4450</td>
<td>0.86 (0.41–1.79)</td>
</tr>
<tr>
<td>Polyunsaturated fats, g/d</td>
<td>4051</td>
<td>4178</td>
<td>4560</td>
<td>4472</td>
<td>0.69 (0.28–1.65)</td>
</tr>
<tr>
<td>Saturated fats, g/d</td>
<td>4424</td>
<td>4257</td>
<td>4065</td>
<td>4515</td>
<td>1.46 (0.67–3.21)</td>
</tr>
<tr>
<td>Fiber, g/d</td>
<td>4302</td>
<td>4090</td>
<td>4335</td>
<td>4534</td>
<td>3.31 (1.44–7.60)</td>
</tr>
<tr>
<td>Folate, μg/d</td>
<td>4387</td>
<td>4253</td>
<td>4246</td>
<td>4375</td>
<td>2.94 (1.28–6.9)</td>
</tr>
<tr>
<td>Iron, mg/d</td>
<td>4320</td>
<td>4181</td>
<td>4261</td>
<td>4499</td>
<td>2.49 (1.03–6.00)</td>
</tr>
<tr>
<td>Magnesium, mg/d</td>
<td>4412</td>
<td>4147</td>
<td>4302</td>
<td>4400</td>
<td>1.38 (0.63–3.02)</td>
</tr>
<tr>
<td>Manganese, mg/d</td>
<td>4098</td>
<td>4277</td>
<td>4418</td>
<td>4468</td>
<td>0.57 (0.26–1.26)</td>
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<tr>
<td>Niacin, mg/d</td>
<td>4017</td>
<td>4281</td>
<td>4473</td>
<td>4490</td>
<td>0.67 (0.27–1.65)</td>
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<tr>
<td>Potassium, mg/d</td>
<td>4403</td>
<td>4279</td>
<td>4249</td>
<td>4330</td>
<td>1.92 (0.88–4.15)</td>
</tr>
<tr>
<td>Protein, g/d</td>
<td>4389</td>
<td>4275</td>
<td>4197</td>
<td>4420</td>
<td>1.02 (0.44–2.36)</td>
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<tr>
<td>Riboflavin, mg/d</td>
<td>4267</td>
<td>4262</td>
<td>4306</td>
<td>4426</td>
<td>2.55 (1.06–6.16)</td>
</tr>
<tr>
<td>Selenium, mg/d</td>
<td>4364</td>
<td>4151</td>
<td>4330</td>
<td>4416</td>
<td>0.56 (0.28–1.23)</td>
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<tr>
<td>Sodium, mg/d</td>
<td>4290</td>
<td>4233</td>
<td>4264</td>
<td>4384</td>
<td>1.72 (0.73–4.03)</td>
</tr>
<tr>
<td>Thiamin, mg/d</td>
<td>3596</td>
<td>4451</td>
<td>4637</td>
<td>4577</td>
<td>2.56 (1.07–6.11)</td>
</tr>
<tr>
<td>Total lipid, g/d</td>
<td>4427</td>
<td>4260</td>
<td>4092</td>
<td>4482</td>
<td>1.33 (0.62–2.94)</td>
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<tr>
<td>Vitamin A, IU/d</td>
<td>4441</td>
<td>4307</td>
<td>4265</td>
<td>4248</td>
<td>2.56 (1.14–5.74)</td>
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<tr>
<td>Vitamin B-6, mg/d</td>
<td>4063</td>
<td>4189</td>
<td>4448</td>
<td>4561</td>
<td>1.59 (0.76–3.32)</td>
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<tr>
<td>Vitamin B-12, mg/d</td>
<td>4385</td>
<td>4415</td>
<td>4345</td>
<td>4116</td>
<td>0.94 (0.41–2.14)</td>
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<tr>
<td>Vitamin C, mg/d</td>
<td>4420</td>
<td>4306</td>
<td>4282</td>
<td>4253</td>
<td>2.29 (1.08–4.88)</td>
</tr>
<tr>
<td>Vitamin E, mg/d</td>
<td>4252</td>
<td>4105</td>
<td>4388</td>
<td>4156</td>
<td>2.75 (1.30–5.83)</td>
</tr>
<tr>
<td>Zinc, mg/d</td>
<td>4218</td>
<td>3947</td>
<td>4543</td>
<td>4553</td>
<td>1.14 (0.54–2.40)</td>
</tr>
</tbody>
</table>

**TABLE 2** Discrete time hazard models of keratotic skin lesion risk in relation to quartiles of nutrient intake (HEALS)
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Gender</th>
<th>Q1 (highest)</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4 (lowest)</th>
<th>P-trend</th>
<th>P-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol equivalents, µg/d</td>
<td>Male</td>
<td>1729</td>
<td>1761</td>
<td>1.35 (0.87–2.10)</td>
<td>1684</td>
<td>0.95 (0.56–1.61)</td>
<td>1433</td>
</tr>
<tr>
<td>Calcium, mg/d</td>
<td>Male</td>
<td>1993</td>
<td>1710</td>
<td>0.79 (0.47–1.34)</td>
<td>1589</td>
<td>1.10 (0.67–1.76)</td>
<td>1315</td>
</tr>
<tr>
<td>Carbohydrate, g/d</td>
<td>Male</td>
<td>1315</td>
<td>1471</td>
<td>1.10 (0.62–1.95)</td>
<td>1732</td>
<td>0.97 (0.57–1.64)</td>
<td>2089</td>
</tr>
<tr>
<td>Cholesterol, mg/d</td>
<td>Male</td>
<td>2240</td>
<td>1774</td>
<td>1.01 (0.61–1.67)</td>
<td>1398</td>
<td>1.08 (0.64–1.82)</td>
<td>1195</td>
</tr>
<tr>
<td>Copper, mg/d</td>
<td>Male</td>
<td>1851</td>
<td>1878</td>
<td>0.81 (0.51–1.28)</td>
<td>1637</td>
<td>0.75 (0.43–1.39)</td>
<td>1241</td>
</tr>
<tr>
<td>Monounsaturated fats, g/d</td>
<td>Male</td>
<td>2082</td>
<td>1679</td>
<td>1.05 (0.60–1.68)</td>
<td>1387</td>
<td>0.82 (0.41–1.63)</td>
<td>1459</td>
</tr>
<tr>
<td>Polyunsaturated fats, g/d</td>
<td>Male</td>
<td>2147</td>
<td>1546</td>
<td>1.26 (0.71–2.23)</td>
<td>1414</td>
<td>1.00 (0.56–1.78)</td>
<td>1501</td>
</tr>
<tr>
<td>Total lipid, g/d</td>
<td>Male</td>
<td>2173</td>
<td>1786</td>
<td>1.11 (0.72–1.76)</td>
<td>1577</td>
<td>1.18 (0.72–1.94)</td>
<td>1244</td>
</tr>
<tr>
<td>Selenium, mg/d</td>
<td>Male</td>
<td>2034</td>
<td>1421</td>
<td>0.90 (0.50–1.63)</td>
<td>1465</td>
<td>0.79 (0.46–1.37)</td>
<td>1687</td>
</tr>
<tr>
<td>Riboflavin, mg/d</td>
<td>Male</td>
<td>2285</td>
<td>1836</td>
<td>0.72 (0.45–1.17)</td>
<td>1478</td>
<td>1.09 (0.66–1.79)</td>
<td>1144</td>
</tr>
<tr>
<td>Magnesium, mg/d</td>
<td>Male</td>
<td>1277</td>
<td>1415</td>
<td>1.12 (0.69–2.17)</td>
<td>1707</td>
<td>0.81 (0.48–1.39)</td>
<td>2212</td>
</tr>
<tr>
<td>Manganese, mg/d</td>
<td>Male</td>
<td>1323</td>
<td>1504</td>
<td>0.77 (0.41–1.44)</td>
<td>1769</td>
<td>0.95 (0.55–1.64)</td>
<td>2011</td>
</tr>
<tr>
<td>Niacin, mg/d</td>
<td>Male</td>
<td>2160</td>
<td>1816</td>
<td>0.72 (0.45–1.14)</td>
<td>1539</td>
<td>0.94 (0.57–1.55)</td>
<td>1092</td>
</tr>
<tr>
<td>Potassium, mg/d</td>
<td>Male</td>
<td>2698</td>
<td>2927</td>
<td>1.05 (0.50–2.19)</td>
<td>2856</td>
<td>1.16 (0.57–2.38)</td>
<td>2173</td>
</tr>
<tr>
<td>Protein, g/d</td>
<td>Male</td>
<td>2208</td>
<td>1777</td>
<td>0.82 (0.51–1.33)</td>
<td>1478</td>
<td>1.09 (0.66–1.79)</td>
<td>1144</td>
</tr>
<tr>
<td>Riboflavin, mg/d</td>
<td>Male</td>
<td>1838</td>
<td>2498</td>
<td>0.82 (0.51–1.31)</td>
<td>2719</td>
<td>1.86 (0.84–4.11)</td>
<td>3276</td>
</tr>
<tr>
<td>Total lipid, g/d</td>
<td>Male</td>
<td>2173</td>
<td>1830</td>
<td>1.25 (0.78–2.01)</td>
<td>1514</td>
<td>1.13 (0.65–1.91)</td>
<td>1090</td>
</tr>
<tr>
<td>Vitamin A, IU/d</td>
<td>Male</td>
<td>2254</td>
<td>1421</td>
<td>1.25 (0.54–2.72)</td>
<td>2568</td>
<td>1.00 (0.39–2.57)</td>
<td>3392</td>
</tr>
<tr>
<td>Vitamin B-6, mg/d</td>
<td>Male</td>
<td>2005</td>
<td>1730</td>
<td>0.83 (0.51–1.34)</td>
<td>1521</td>
<td>0.97 (0.58–1.60)</td>
<td>1360</td>
</tr>
<tr>
<td>Vitamin B-12, mg/d</td>
<td>Male</td>
<td>2144</td>
<td>1680</td>
<td>0.86 (0.58–1.60)</td>
<td>1521</td>
<td>1.00 (0.58–1.70)</td>
<td>1282</td>
</tr>
<tr>
<td>Zinc, mg/d</td>
<td>Male</td>
<td>1996</td>
<td>1746</td>
<td>1.16 (0.68–1.97)</td>
<td>1509</td>
<td>1.56 (0.96–2.53)</td>
<td>1356</td>
</tr>
</tbody>
</table>

1 Models are adjusted for age, water arsenic concentration, BMI, formal education years, years of education, smoking, and follow-up interval. HEALS, Health Effects of Arsenic Longitudinal Study.
their childbearing years (23). The present study suggests that folate, which is an important nutrient during childbearing years, could potentially also affect a woman’s ability to metabolize arsenic (24). Increased intake of nutrients such as calcium (11), vitamin A (10), and B vitamins (9) (including riboflavin) was shown in previous studies to positively affect arsenic metabolism and play a role in oxidative stress pathways. These results are consistent with prior studies that suggest that while males in general have an increased incidence of skin lesions, potentially due to environmental factors such as increased sun exposure, the dose response association between arsenic exposure and skin lesion status is more pronounced in women (25).

The previously conducted research described above provides evidence that dietary factors, nutritional intake, and gender may influence arsenic metabolism and/or adverse health outcomes associated with arsenic exposure. In the present study, several of the aforementioned nutrients were also found to have inverse associations with skin lesion risk, particularly for more severe keratotic skin lesions. Whereas nutrient deficiency seemed to be associated with higher risk of nonkeratotic skin lesions in men, the more severe keratotic skin lesions showed higher risk in nutrient-deficient women. Although studies generally have shown that men may be more susceptible to skin lesions overall in this population (13,15,26), the present study suggests that women’s ability to metabolize arsenic may be more profoundly affected by nutritional deficiencies, particularly in the case of more severe keratotic skin lesions.

In general, utilization of FFQ data is a limitation, because it is not an ideal methodology for quantifying intake of food or various nutrients (27). Certain nutrients have been shown to have poor concordance between FFQ-measured intake and intake based on diary or recall methods, including folate, fiber, protein, potassium, retinol, and vitamin E (28,29). This could potentially introduce bias into the results of our study, because several of these nutrients had significant associations with skin lesions. However, the FFQ utilized here was specifically designed and validated for the HEALS population, which has a diet that is comprised of mainly locally and seasonally available foods (16). Additionally, although the FFQ is limited in its ability to quantify exact intake of nutrients, it is a useful and efficient methodology for ranking individuals from highest to lowest based on their nutrient intake.

The use of the USDA nutrient database is another potential limitation to the present study, because this database does not specifically reflect the diets of the Bangladeshi population. Although the RDA database for India may provide a closer approximation of the nutrient intake for this population, it is much less comprehensive than the USDA nutrient database. Therefore, results utilizing the USDA nutrient database are presented here.

Another potential limitation in this study is that we conducted multiple comparisons in our analyses and it is possible that some of the significant results in this study occurred by chance. However, because the comparisons were all prespecified, we chose not to correct for multiple testing. The present study was prospective in nature; therefore, it is unlikely that our results were affected by recall bias, selection bias, or reverse causality. To control for confounding, we included variables to account for socioeconomic status (via education), water arsenic concentrations, BMI, and smoking status, all of which could potentially have independent associations with skin lesion status and be associated with diet.

The present study provides important information regarding the role of nutrient intake in susceptibility to arsenic-related toxicity, in particular, keratotic skin lesions. Previous studies have established that arsenic exposure is necessary for skin lesion incidence; however, skin lesion incidence also occurs at various doses of arsenic exposure. In this study, we were able to assess the impact of nutritional intake on a population ubiquitously exposed to varying levels of arsenic via drinking water. Holding this exposure constant across the population, we were able to detect the impact of nutritional intake on arsenic toxicity. Although the most effective way to reduce arsenic-related toxicity in this population is to eliminate arsenic consumption through the drinking water, it is possible that a large proportion of the most serious keratotic skin lesions in women can be prevented or attenuated by providing diets more rich in nutrients, including calcium, fiber, vitamin E, and retinol. This work suggests that the impact of nutrient intake on skin lesion susceptibility is highly complex and varies by gender and lesion severity. Further research is necessary to understand the increased susceptibility to severe keratotic skin lesions experienced by women in this population that may be nutrient deficient.

Acknowledgments
H.A. designed research and directed its implementation; S.M., M.A., B.P., and H.A. designed and implemented the study’s analytic strategy and helped prepare the Materials and Methods and Discussion portions of the text; and critical reviews and revisions were conducted by S.M., M.A., B.P., H.A., Y.C., T.I., A.A., and F.P. All authors read and approved the final manuscript.

Literature Cited


