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Nutritional Factors and Susceptibility to Arsenic-Caused Skin Lesions in West Bengal, India

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There has been widespread speculation about whether nutritional deficiencies increase the susceptibility to arsenic health effects. This is the first study to investigate whether dietary micronutrient and macronutrient intake modulates the well-established human risk of arsenic-induced skin lesions, including alterations in skin pigmentation and keratoses. The study was conducted in West Bengal, India, which along with Bangladesh constitutes the largest population in the world exposed to arsenic from drinking water. In this case–control study design, cases were patients with arsenic-induced skin lesions and had < 500 μg/L arsenic in their drinking water. For each case, an age- and sex-matched control was selected from participants of a 1995–1996 cross-sectional survey, whose drinking water at that time also contained < 500 μg/L arsenic. Nutritional assessment was based on a 24-hr recall for major dietary constituents and a 1-week recall for less common constituents. Modest increases in risk were related to being in the lowest quintiles of intake of animal protein [odds ratio (OR) = 1.94; 95% confidence interval (CI), 1.05–3.59], calcium (OR = 1.89; 95% CI, 1.04–3.43), fiber (OR = 2.20; 95% CI, 1.15–4.21), and folate (OR = 1.67; 95% CI, 0.87–3.2). Conditional logistic regression suggested that the strongest associations were with low calcium, low animal protein, low folate, and low fiber intake. Nutrient intake was not related to arsenic exposure. We conclude that low intake of calcium, animal protein, folate, and fiber may increase susceptibility to arsenic-caused skin lesions. However, in light of the small magnitude of increased risks related to these dietary deficiencies, prevention should focus on reducing exposure to arsenic. *Key words:* **arsenic, case–control study, environmental health, India, nutritional susceptibility, skin lesions.** *Environ Health Perspect* **112:1104–1109 (2004). doi:10.1289/ehp.6841 available via** *http://dx.doi.org/* **[Online 13 April 2004]**

A public health crisis has unfolded in West Bengal, India, and neighboring Bangladesh. Large populations in nine districts of West Bengal consume groundwater contaminated with inorganic arsenic that has leached from underground natural geologic formations. An estimated 6 million people in West Bengal are presently drinking water contaminated with arsenic > 50 μg/L in an area of 38,865 km^2 (Chowdhury et al. 2001). In some affected villages, people have been found to consume groundwater with arsenic concentrations > 1,000 μg/L (Guha Mazumder et al. 1998a).

Skin abnormalities, such as pigmentation and keratoses, have long been known to be caused by chronic ingestion of inorganic arsenic. A few studies have resulted in hypotheses that the prevalence of arsenic-induced skin lesions and other arsenic-caused diseases might be increased with malnutrition (National Research Council 1999). In southwestern Taiwan, inhabitants were reported to have poor nutritional and low socioeconomic status (Yang and Blackwell 1961); their reported diet was adequate in calories and high in carbohydrates but low in protein and extremely low in fat. In another study on the same population, Hsueh et al. (1995) reported that undernourishment—indexed by a high consumption of dried sweet potatoes as a staple food—was associated with an increased prevalence of arsenic-induced skin cancer. It has been suggested that low intake of micronutrients in this population may have led to a greater susceptibility to carcinogenesis (Engel and Receveur 1993). More detailed nutritional data, although still lacking quantities of food consumed, were presented in a study in Taiwan that found increased risks of blackfoot disease (a peripheral vascular disease attributed to arsenic in drinking water) associated with undernourishment (Chen et al. 1988). Thus, the investigation of potential increased human susceptibility to arsenic-caused disease related to nutrition have been limited in nature and largely confined to Taiwan, although a small study in northern Chile reported arsenic-related skin lesions in a well-nourished population (Smith et al. 2000). Experimental studies concerning diet and arsenic in animals have yielded interesting findings (Vahter and Marafante 1987), but the implications for humans are not clear because humans are much more sensitive to arsenic-caused disease than experimental animals and they metabolize arsenic differently (National Research Council 1999, 2001).

Arsenic metabolism involves methylation, so nutritional factors that might influence the toxicity of arsenic include methyl group donors such as methionine, the B group of vitamins, and folate (National Research Council 1999). Low intake of certain micronutrients, including zinc and selenium, might also increase arsenic-caused disease risks (Engel and Receveur 1993; Levender 1977). The main objective of the present study was to determine if increased risks of arsenic skin lesions are associated with inadequate nutrition (or low intake of specific nutrients) among individuals who consumed < 500 μg/L arsenic in their drinking water. We focused on participants who consumed drinking water from sources containing < 500 μg/L inorganic arsenic because enhanced susceptibility may be more apparent in those who developed disease as a consequence of relatively low arsenic intake, since those with very high intake may succumb to effects whatever their diet is. This is the first study with quantitative evaluation of micro- and macronutrients in the diet of persons with and without skin lesions. Information concerning dose–response relationships between skin lesions and arsenic water concentrations in this study population has been published separately (Haque et al. 2003).

Materials and Methods

Source population. A prevalence survey was conducted in 1995–1996 in 57 villages of South 24-Parganas, a rural district located 30 km south of Calcutta (Guha Mazumder et al. 1998a). The arsenic levels in the water

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from tube wells of the region ranged from nondetectable to 3,400 μg/L. Among the approximately 7,000 individuals who had participated in the previous survey, 415 individuals with classic arsenic-induced skin lesions were identified. This nested case–control study is based on the survey participants who were exposed to < 500 μg/L inorganic arsenic from their primary water source at that time. A total of 4,185 (2,160 females and 2,025 males) of the original survey participants were in this category, and 256 of them had skin lesions.

Case and control selection. The case–control study protocol was approved by the institutional review boards of both the Institute of Post Graduate Medical Education and Research, Calcutta, and the University of California, Berkeley. Informed consent was obtained before administering the questionnaire.

Cases with skin lesions were selected from the cross-sectional survey and included all individuals who had been diagnosed to have arsenic-induced skin lesions and whose main water source was found to contain < 500 μg/L arsenic at the time of the 1995–1996 survey. Of the target 265 cases (100 females and 165 males), 174 had pigmentation changes, 15 had keratoses, and 76 had both types of lesions, at the time of the survey in 1995–1996. Hyperpigmentation was identified as raindrop-shaped discolored spots, diffuse dark brown spots, or diffuse darkening of the skin on the limbs and trunk. Simple keratosis was identified as bilateral thickening of the palms and soles, and nodular keratosis was identified as small protrusions appearing on the palms and soles, and occasionally also on the dorsum of the hands and feet or on the legs (Guha Mazumder et al. 1998a). Some of the cases no longer had apparent skin lesions when examined in 1998–2000 (Haque et al. 2003) but were retained as cases for the nutritional investigation.

Controls were selected from survey participants who did not have skin keratoses or hyperpigmentation when seen during the 1995–1996 survey and whose main tube-well water source, like the cases, contained < 500 μg/L arsenic. For each case, one control matched by age (± 5 years) and sex was randomly identified from all eligible noncases. Replacement controls were selected for controls who had died, could not be located, or did not wish to participate.

Field investigations. Participants were visited in their homes between April 1998 and January 2000. Demographic and socioeconomic data were ascertained through a structured questionnaire. Education was categorized into three categories: *a*) no formal education, *b*) primary, and *c*) secondary or higher education. In India, socioeconomic status is often measured by type of house, which is correlated with household economic status (Mishra et al. 1999). Homes were differentiated between those built of high-quality materials, such as concrete or brick, and those built of lower quality materials, such as mud or thatched natural fibers. Information on a participant's diet was obtained by interview with the woman in charge of food preparation for each household.

Assessment of nutritional status. Weight and height were measured, and body mass index (BMI) was calculated (Quetelet's index: weight/height^2 in kilograms per meters squared). The ratio of the subjects' observed weight to the standard or expected weight was calculated using standard weights obtained from a chart of desirable weights (listed by age, height, and sex) used by the Life Insurance Corporation of India Ltd. (a Government of India enterprise). Food intake was ascertained by a detailed questionnaire based primarily on 24-hr recall. The "senior" woman, who in this population directs preparing food for the family, was interviewed. She was often the mother in the family, or else the eldest daughter-in-law of the family. Raw food materials were weighed whenever the participating woman was unable to state the actual weight of the food used. To estimate the amounts and volumes of food consumed, plates and cups of various sizes were shown to the participating woman during the interview. Dish volumes were standard amounts listed by the National Institute of Nutrition, Hyderabad, for use in diet surveys (Gopaldas and Seshadri 1987).

The participating woman was questioned about each meal, from the previous day's lunch through the breakfast on the day of the interview. All food items used to prepare each meal were noted, and their weights in grams were recorded in the questionnaire. The volume of each cooked food was assessed by questioning the senior woman using the standard cups and plates. From the total cooked food, the portion given to the individual participant was also assessed by cups and plates exhibited to the woman. Standard-size spoons were used to assess the intake of sugar and oil. Participants who worked in the fields often carried food from home. If not, then the participant was questioned about purchased food items. Each participant was also questioned directly about his or her food likes and dislikes and asked to briefly confirm what was reported by the woman of the house. Questions were asked about the weekly consumption of meat, fish, eggs, milk, and fruit because these items were not consumed on a daily basis. A 1-week intake of these food items was then divided by 7 to compute the average intake per day. Individual intake in terms of each raw food item (rice, legumes, potato) was calculated by the formula $F = (P/Q) \times R$, where F is the individual intake of a raw food item by the study participant from a particular preparation; P is the amount in grams of the total raw food ingredient, F, used for cooking this preparation; Q is the total volume in milliliters of the cooked food preparation; and R is the volume in milliliters of the cooked food preparation consumed by the study participant.

The total intake (24 hr) of each nutrient (carbohydrate, protein, vitamins), and calorie consumption were calculated using a spreadsheet program. For this purpose, a detailed database was prepared for the nutrient composition per 100 g of raw food items.

The protein, fat, carbohydrate, energy, fiber, mineral (calcium, phosphorus, iron, and zinc), and vitamin (thiamin, riboflavin, niacin, vitamin B6, folate, carotene, retinol, and vitamin C) composition of raw foods were obtained from information published by the National Institute of Nutrition, India (Gopalan et al. 1996). As in other countries, selenium intake in the diet is highly variable depending on the soil where food is grown. Hence, it was not possible to assess selenium intake in this study.

Food composition (nutritive values) for ready-to-eat items (e.g., biscuits, health beverages, skimmed milk) was obtained from the food packages. Food composition of items not typically prepared in homes was averaged from information obtained from the owners of five shops. Vitamin supplements are not used in the study population.

Assessment of arsenic exposure. Water samples were collected from all functioning tube wells used by participants at least 6 months in the past 20 years. Samples were collected from tube wells used earlier if they were still in use. For the closed tube wells, historical arsenic measurements were obtained. We collected samples from approximately 800 functioning tube wells in the 21 villages combined. Total water arsenic was measured by flow injection analysis using fluorescence detection with inline photo oxidation and continuous hydride generation (Attalah and Kalman 1991). Details of the water collection and arsenic exposure assessment are published elsewhere (Haque et al. 2003).

Statistical methods. Information acquired from the diet survey of participants was used to elucidate their nutrient intake per day. The mean value of the total energy consumption, protein from animal and vegetable sources, fat from animal and vegetable sources, carbohydrates, fiber, calcium, iron, phosphorus, zinc, carotene, retinol, thiamin, riboflavin, niacin, vitamin B6, folate, and vitamin C was computed. We conducted two-tailed paired *t*-tests for the difference in average nutrient intake between cases and controls. Information about tube-well use at each residence and work site, and the results of the arsenic measurements were used to construct arsenic exposure

histories. We estimated average and peak arsenic exposure. Annual average water concentrations were first calculated for participants for each calendar year based on the measured water arsenic concentrations for each tube well used in that year and on the fraction of their drinking water participants obtained from that source in that year. We defined the peak arsenic exposure as the maximum 1-year average concentration of arsenic in drinking water (Haque et al. 2003). Examination of the relationship between nutrient intake and peak arsenic concentrations included testing for differences in nutrient intake between those with the highest and lowest peak arsenic concentrations, and calculation of correlations between arsenic and nutrients in controls that reflect the source population in a case–control study. The nutrient intakes were next stratified in quintiles of the distribution of the controls, and odds ratios (ORs) with 95% confidence intervals (95% CIs) were estimated for each level, taking the highest quintile as the reference group. Tests for trend were based on the chi-square distribution using the median of each quintile range.

Case and control nutrient intakes were compared with the recommended dietary allowances (RDA) for India. Both cases and controls were stratified on sex for comparison of the nutrient intakes with the respective RDAs, and the information for the sexes was then pooled. The proportions of cases and controls with nutrient intakes below the RDAs were then compared.

Selected nutrients (those found to have the strongest univariate differences between cases and controls) were assessed by conditional logistic regression analysis, after standardization of each variable so that one unit represented 1 SD. The coefficients for each factor were then used to derive the OR associated with an increase in 1 SD for each factor. The analysis was first conducted for each primary variable on its own, the primary variables along with each potential confounding variable, and then with peak arsenic levels in addition to potential confounders.

The confounding variables assessed were BMI, house construction (concrete house compared with mud house as an indicator variable), and education in three categories (college and/or secondary education, primary education, and no formal education). Age and sex were matching variables implicit in the conditional logistic regression.

Results

Of the 265 potentially eligible cases and 256 selected controls, 32 cases (12%) and 38 controls (14%) had moved outside the study region or could not be located. A further 26 cases (10%) and 14 controls (5%) were too ill to participate, had died, or refused. Because of known animosity toward the field team, 15 cases (6%) identified in the cross-sectional survey living in two adjacent hamlets were not invited to participate (no controls lived in such areas). A total of 192 cases (72%) participated in the study. Initially, 213 controls (80%) were selected and interviewed. Excluding those who had moved outside the study region or could not located, the participation rate was 88% for cases and 94% for controls. For the purpose of the nutrition study, the extra matched controls, who had been selected and interviewed before it was known their cases could not participate, were excluded from the analysis, leaving 192 matched case–control pairs. When a control could not participate, for whatever reason, a replacement control was selected.

Cases and controls were similar in terms of general characteristics (Table 1). The mean BMI was approximately 19 kg/m^2 in both groups. On average, cases were 88.7% and controls were 89.3% of Indian standard weights. The distribution of cases and controls in relation to arsenic exposure showed that significantly more cases were exposed to increased arsenic levels of ≥ 100 µg/L ($p < 0.001$; Table 1). The mean peak arsenic exposure was 329.9 µg/L in cases and 163.2 µg/L in controls ($p < 0.001$). We found no significant difference in the intake of each nutrient among controls exposed to low (< 100 µg/L) versus high (≥ 100 µg/L) peak arsenic levels (Table 2).

Table 3 presents the average nutrient intake of cases and controls. The overall energy intake in cases (9,210 kJ) was a little lower than for controls (9,552 kJ). The strongest findings for specific nutrients in comparing mean differences between cases and controls involved animal protein, fiber, calcium, and vitamin C. Both cases and controls were stratified on sex for comparison of the nutrient intakes with the respective RDAs, and the information for the sexes was then pooled. RDAs of Indian males and females are presented in Table 3 for reference. In both males and females, iron, zinc, retinol, riboflavin, and vitamin B6 consumption were below the RDAs for at least 80% of both cases and controls. For retinol, 100% of both cases and controls were below the RDA of 600 µg/day.

Comparing cases and controls showed little difference in the percentage below the RDA for most nutrients (Table 3). However, for calcium, 53% of cases were below the RDA, compared with 39% of controls ($p = 0.04$).

Table 4 presents the data on intake of each nutrient stratified into quintiles. ORs and 95% CIs were computed for each stratum, using the highest quintile as the reference group. The strongest trends in ORs were for animal protein, fiber, calcium, folate, and vitamin C. None of these nutrients was related to arsenic exposure in the correlation analysis (data not shown). At the lowest quintile of intake, the ORs were as follows: animal protein (1.94), fiber (2.20), calcium (1.89), folate (1.67), and vitamin C (1.50). Interestingly, there was little, if any, evidence of associations with zinc, retinol, and vitamin B6, each of which have been postulated to play a possible role in skin lesion risk or impact on arsenic methylation (National Research Council 1999).

Table 1. Demographic and general information for cases ($n = 192$) and controls ($n = 192$).

	Cases No. (%)	Controls No. (%)	*p*-Value
Sex			
Male	119 (62)	119 (62)	
Female	73 (38)	73 (38)	
Education			
College	8 (4.2)	11 (5.7)	
Secondary	27 (14.1)	33 (17.2)	
Primary	97 (50.5)	96 (50.0)	
No formal education	59 (30.7)	48 (25.0)	
Missing	1 (0.5)	4 (2.1)	0.53[a]
House			
Concrete/mixed	30 (15.6)	24 (12.5)	
Mixed quality materials	63 (32.8)	68 (35.4)	
Mud/thatched	99 (51.6)	96 (50.0)	
Missing	0 (0.0)	4 (2.1)	0.65[a]
Peak arsenic (µg/L)			
0–99	12 (6.3)	74 (38.5)	
100–399	129 (67.2)	95 (49.5)	
≥ 400	51 (26.6)	23 (12.0)	< 0.001[a]
Group means			
Age (years)	41.5	41.3	0.88
Height (m)	1.55	1.55	0.96
Weight (kg)	45.8	46	0.94
BMI	18.8	18.9	0.82
Percent of ISW	88.7	89.3	0.83

ISW, Indian standard weight.
[a]Chi-square tests.

Table 5 presents the results of the multivariate analysis by conditional logistic regression. Each of the variables was first standardized by dividing all values by the corresponding SDs given in Table 5. The unadjusted ORs represent the impact of an increase of 1 SD in the factor. For example, for an increase in fiber intake of 3.5 g, the SD, the estimate of the OR is that the risk of skin lesions would reduce from 1 to 0.80 (95% CI, 0.65–0.98). In the multivariate model that includes peak arsenic levels (full model), the estimate is 0.86 (0.67–1.09). Although there remains some apparent potential effects of fiber, calcium, animal protein, and folate, adjustment in the full model brings the OR for iron and vitamin C closer to 1. For example, the OR for vitamin C rises from 0.82 to 0.91. This evidence suggests that the associations with animal protein, calcium, fiber, and folate intake might be real, but that the relationship with vitamin C might be secondary (i.e., due to confounding).

In addition to key independent primary variables, we also considered BMI, housing status, and education level of cases and controls as potentially confounding variables. We did not find significant differences between cases and controls in any of these variables. As shown in Table 5, adjustment for these variables had negligible effect.

Discussion

The overall objective of the present study was to identify nutritional factors that might modify susceptibility to arsenic-induced skin lesions. Study participants were selected from a previous cross-sectional survey in rural villages of the South 24-Parganas district who had been drinking water contaminated with < 500 µg/L arsenic from tube wells in the region. It was thought that enhanced susceptibility might be more apparent in those with < 500 µg/L arsenic in drinking water, selected from within a source population where concentrations often exceed 1,000 µg/L (Guha Mazumder et al. 1998a).

An important finding of the present study was that deficiency in some nutritional factors may increase the risk of arsenic-induced skin lesions. The strongest evidence was for low intake of animal protein, calcium, fiber, folate, and vitamin C. ORs increased to nearly 2.0 for those in the lowest quintile of intake for animal protein, calcium, and fiber compared with those in the highest quintile (Table 4). In the multivariate analysis including arsenic, the relationship with vitamin C diminished, suggesting that it was a secondary association, thus leaving animal protein, calcium, folate, and fiber as the nutrients with the greatest potential effects. It is not clear why animal protein should be important with regard to skin lesions. Fiber intake also lacks explanation. It is possible that fiber might reduce arsenic absorption from the gastrointestinal tract, but we know of no evidence for this. A large fraction of fiber intake in this population comes from rice intake, but because rice is the main source of carbohydrate for these people, and carbohydrate intake did not differ between cases and controls, reduced risks cannot be attributed to rice intake.

In order to identify the factors affecting apparent absorption of copper and zinc in vegetarian subjects, Agte et al. (1994) estimated percent absorption of copper and zinc during six metabolic experiments, each of 2 weeks' duration carried out on six healthy young males. They concluded that riboflavin, cellulose, milk proteins, oxalates, and zinc enhanced copper absorption, whereas phosphorus, niacin, calcium, and pulse protein inhibited the absorption of copper. We do not know whether calcium and other nutrients played a similar role in inhibiting arsenic absorption in our population.

Regarding vegetables, it is possible that a variety of constituents might be involved. As noted, vitamin C itself showed some relationship with risk (Table 4), and there was some evidence of a relationship with carotene (OR increasing to 1.57 in the lowest intake quintile; trend test $p = 0.05$). However, because

Table 2. Nutrient intake (mean ± SE) in relation to peak arsenic exposure[a] in the control population.

Nutrient	Peak arsenic < 100 µg/L ($n = 74$)	Peak arsenic ≥ 100 µg/L ($n = 118$)	p-Value[b]
Energy (kJ)	9,946 ± 393	9,326 ± 282	0.28
Protein, animal (g)	10.3 ± 0.8	10.4 ± 0.7	0.88
Protein, vegetable (g)	48.1 ± 2.2	44.2 ± 1.5	0.14
Fat, animal (g)	3.6 ± 0.4	4.3 ± 0.4	0.21
Fat, vegetable (g)	23.0 ± 1.9	22.2 ± 1.5	0.74
Carbohydrate (g)	472 ± 20.8	440 ± 13.7	0.17
Fiber (g)	5.9 ± 0.4	5.5 ± 0.3	0.53
Calcium (mg)	520 ± 32	524 ± 39.6	0.94
Iron (mg)	15.4 ± 0.9	13.9 ± 0.8	0.20
Phosphorus (mg)	1,206 ± 55	1,144 ± 41.8	0.37
Zinc (mg)	9.6 ± 0.4	9.1 ± 0.3	0.33
Carotene (µg)	4,641 ± 922	3,877 ± 767	0.53
Retinol (µg)	47.8 ± 6.5	58.1 ± 6.9	0.27
Thiamin (mg)	1.56 ± 0.1	1.50 ± 0.05	0.43
Riboflavin (mg)	0.64 ± 0.03	0.65 ± 0.03	0.89
Niacin (mg)	23.2 ± 1.0	21.7 ± 0.7	0.22
Vitamin B6 (mg)	1.34 ± 0.1	1.27 ± 0.05	0.36
Folate (µg)	181 ± 12	169 ± 10.7	0.49
Vitamin C (mg)	131 ± 14.3	121 ± 10.9	0.57

[a]Peak arsenic exposure defined as maximum 1-year average concentration of arsenic in drinking water (Haque et al. 2003); the arsenic cutoff was determined as the closest round number to the median of the control distribution. [b]Two-tailed t-test.

Table 3. Comparison of nutrient intake between cases and controls and comparison of percentages of cases and controls below RDA values for India.

Nutrient	Average Case	Average Control	Average SD	Average p-Value[a]	Percent < RDA Case	Percent < RDA Control	Percent < RDA p-Value[b]	RDA Male	RDA Female
Energy (kJ)	9,210	9,552	756	0.25	68.2	62	0.35	12,029	7,845
Protein, animal (g)	8.70	10.40	6.98	0.02	NA	NA		NA	NA
Protein, vegetable (g)	44.1	45.8	17.1	0.29	NA	NA		NA	NA
Protein, total (g)	52.8	56.2	19.6	0.11	59.9	52.6	0.29	60	50
Fat, animal (g)	3.7	4.1	4.3	0.45	NA	NA		NA	NA
Fat, vegetable (g)	20.8	22.7	14.8	0.22	NA	NA		NA	NA
Fat, total (g)	24.5	26.8	16.1	0.22	45.3	41.2	0.55	20	20
Carbohydrate (g)	443	453	161	0.46	NA	NA	NA	NA	NA
Fiber (g)	4.8	5.7	3.5	0.02	NA	NA	NA	NA	NA
Calcium (mg)	439	520	336	0.02	53.1	39.1	0.04	400	400
Phosphorus (mg)	1,091	1,170	439	0.06	1	0.52	0.67	400	400
Iron (mg)	13.1	14.6	7.1	0.07	97.9	95.3	0.31	28	30
Zinc (mg)	9.1	9.2	3.3	0.45	97.9	93.3	0.10	15	15
Carotene (µg)	3,024	4,068	7,283	0.17	75	68.8	0.32	NA	NA
Retinol (µg)	49.6	54.5	66.4	0.45	100	100	NA	600	600
Thiamine (mg)	1.46	1.52	0.57	0.22	29.7	29.2	0.93	1.4	0.9
Riboflavin (mg)	0.60	0.65	0.33	0.31	96.9	95.3	0.56	1.6	1.1
Niacin (mg)	21.7	22.3	8.1	0.40	22.9	22.4	0.93	18	12
Vitamin B6 (mg)	1.26	1.30	0.53	0.45	91.7	89.1	0.53	2	2
Folate (µg)	153	173	103	0.06	27.6	22.4	0.39	100	100
Vitamin C (mg)	97.4	124	116	0.04	25.5	22.4	0.60	40	40

For protein and fat, RDAs are available for totals only.
[a]Based on two-tailed t-test. [b]Based on chi-square test.

both of these associations are weaker than for fiber, it is perhaps more likely that a variety of nutrients in vegetables would be involved.

It is noteworthy that there was widespread undernutrition found in this study, as judged by Indian RDA values (Table 3). Although this study suggests that risks are associated with low intake of certain dietary constituents, it is of course possible that the effects are confined to certain unidentified micronutrients within them. Large proportions of cases and controls were below the RDAs for most nutritional factors. However, the only major difference in RDA comparisons between cases and controls was for calcium. For some factors, in particular with retinol, all cases and controls had intakes below the RDA of 600 μg. However, severely low intake of retinol may be compensated by intake of carotene, some of which is converted to retinol. For iron, riboflavin, and vitamin B6, most of both cases and controls fall below the RDAs, too. This raises the possibility that, if there was deficiency in all participants for a nutrient, the study might not pick up its association with risk. However, even though intake was generally low, there was a range of intake for each of these nutrients. There was no evidence of increased risk in the lowest quintile of intake of retinol (Table 4). There was also little evidence of increased risks for the lowest quintile intakes of riboflavin and vitamin B6.

Nutritional deficiency is common in rural India. For example, Pushpamma et al. (1982) investigated the food intake and nutrient adequacy of a rural population in Andhra Pradesh, India. They reported that the intake of green leafy vegetables, milk, animal protein foods, and oils was very low in all age groups. The intake of vitamin A and ascorbic acid was grossly inadequate in every group. Bamji (1983) reported on nutrition surveys in eight states of India conducted by the National Nutrition Bureau of India; although the average energy intake was adequate, > 50% of households surveyed consumed less than the RDA of energy. The corresponding values for the present study were 68% in cases and 62% in controls. The average intakes of vitamin A and riboflavin were also inadequate.

The greatest weakness of the present study was the cross-sectional ascertainment of dietary intake after the development of skin lesions. We presumed a relationship existed between current and past dietary intakes. There may be trends over time with changes in nutrient intake, but the assumption was that participants will more or less maintain their intakes in rank order. For example, if a participant currently has a high fiber intake relative to other participants, the implicit assumption was that they would have had a relatively high intake compared with other participants during the period of skin lesion causation. However, if there were a change in diet due to the presence of skin lesions, then one possibility would be a reduction in total caloric intake due to loss of appetite.

The average caloric intake of cases (9,210 kJ/day) was not much lower than that for controls (9,552 kJ/day). BMI did not differ between cases and controls. Hence, the findings cannot be attributed to an overall reduction in food intake in those with skin lesions. Nevertheless, it is possible that the presence of skin lesions resulted in dietary changes that contributed to some of the dietary differences observed here.

Rural populations in West Bengal have relatively stable diets. Even so, individual dietary intake may vary from day to day, so the last 24 hr may not necessarily be representative for each individual. However, in an epidemiologic study such as this, the inference is about groups and subgroups of participants, with pooling or averaging of individual data. For these reasons, 24-hr recall of dietary

Table 5. Effects of nutrients on ORs for 1 SD change in each nutrient, before and after adjustment for socioeconomic variables, BMI, and arsenic.

Nutrient	SD	Unadjusted OR (95% CI)	Adjusted[a] OR (95% CI)	Adjusted[b] OR (95% CI)
Fiber (g)	3.5	0.80 (0.65–0.98)	0.80 (0.65–0.99)	0.86 (0.67–1.09)
Calcium (mg)	336	0.76 (0.60–0.95)	0.75 (0.59–0.95)	0.80 (0.64–1.04)
Animal protein (g)	6.98	0.78 (0.63–0.97)	0.78 (0.62–0.99)	0.83 (0.64–1.09)
Vitamin C (mg)	116	0.82 (0.68–1.00)	0.81 (0.66–0.99)	0.91 (0.71–1.16)
Phosphorus (mg)	439	0.81 (0.65–1.02)	0.81 (0.64–1.02)	0.89 (0.68–1.17)
Iron (mg)	7.1	0.82 (0.66–1.03)	0.81 (0.64–1.02)	0.95 (0.72–1.26)
Folate (μg)	103	0.80 (0.65–0.99)	0.79 (0.64–0.98)	0.86 (0.65–1.13)
Peak arsenic (μg)		1.01 (1.00–1.01)		
BMI (high vs. low)		1.83 (0.68–4.96)		
Education, primary versus no formal education		1.00 (0.66–1.52)		
Education, college versus no formal education		0.73 (0.43–1.23)		
Concrete house versus mud construction		0.98 (0.66–1.45)		

[a]Adjusted for BMI, education status, and housing status. [b]Adjusted for peak arsenic, BMI, education status, and housing status.

Table 4. ORs (95% CIs) by quintiles of nutrient intake with highest intake quintile (quintile 1) as the reference group.

Nutrient	OR (highest to lowest quintile) 1	2	3	4	5	Test for trend[a]
Energy (kJ)	1	1.56 (0.83–2.91)	0.90 (0.46–1.76)	0.93 (0.48–1.82)	1.52 (0.81–2.83)	0.53
Protein, animal (g)	1	0.74 (0.37–1.48)	1.35 (0.71–2.56)	1.06 (0.55–2.05)	1.94 (1.05–3.59)	0.001
Protein, vegetable (g)	1	1.35 (0.72–2.52)	1.06 (0.55–2.01)	0.76 (0.39–1.50)	1.40 (0.75–2.60)	0.72
Fat, animal (g)	1	1.30 (0.68–2.5)	1.33 (0.69–2.56)	1.44 (0.75–2.75)	1.43 (0.75–2.73)	0.16
Fat, vegetable (g)	1	1.17 (0.62–2.22)	1.32 (0.70–2.47)	0.79 (0.40–1.55)	1.29 (0.69–2.40)	0.75
Carbohydrate (g)	1	0.49 (0.26–0.92)	0.49 (0.26–0.92)	0.52 (0.28–0.98)	0.91 (0.51–1.63)	0.72
Fiber (g)	1	1.23 (0.62–2.46)	1.85 (0.95–3.58)	1.52 (0.77–2.99)	2.20 (1.15–4.21)	0.002
Calcium (mg)	1	0.58 (0.29–1.17)	0.86 (0.45–1.65)	0.92 (0.48–1.75)	1.89 (1.04–3.43)	0.001
Iron (mg)	1	1.40 (0.73–2.68)	0.96 (0.48–1.89)	1.54 (0.81–2.93)	1.60 (0.85–3.02)	0.07
Phosphorus (mg)	1	2.32 (1.19–4.51)	1.70 (0.86–3.36)	1.20 (0.59–2.46)	2.26 (1.17–4.38)	0.14
Zinc (mg)	1	1.21 (0.65–2.23)	0.74 (0.39–1.43)	0.74 (0.39–1.43)	1.18 (0.64–2.17)	0.86
Carotene (μg)	1	1.17 (0.60–2.31)	1.72 (0.90–3.29)	1.50 (0.78–2.90)	1.57 (0.82–3.01)	0.05
Retinol (μg)	1	1.40 (0.73–2.68)	1.54 (0.81–2.93)	1.47 (0.77–2.81)	1.10 (0.57–2.14)	0.71
Thiamine (mg)	1	1.35 (0.72–2.54)	0.92 (0.48–1.77)	0.97 (0.50–1.87)	1.35 (0.72–2.52)	0.62
Riboflavin (mg)	1	0.95 (0.49–1.84)	0.87 (0.45–1.69)	1.18 (0.62–2.26)	1.44 (0.77–2.68)	0.07
Niacin (mg)	1	1.13 (0.61–2.11)	0.97 (0.52–1.84)	0.63 (0.32–1.24)	1.26 (0.68–2.32)	0.96
Vitamin B6 (mg)	1	1.51 (0.81–2.82)	0.66 (0.33–1.33)	1.15 (0.60–2.18)	1.41 (0.76–2.64)	0.48
Folate (μg)	1	0.99 (0.49–1.99)	1.79 (0.93–3.43)	1.79 (0.93–3.43)	1.67 (0.87–3.20)	0.006
Vitamin C (mg)	1	1.03 (0.51–2.07)	2.33 (1.23–4.43)	1.66 (0.85–3.22)	1.50 (0.77–2.92)	0.04

[a]*p*-Value based on chi-square test for trend.

intake is a useful method for assessing nutrition in population studies such as this.

Yang and Blackwell (1961) conducted a study of the diet and environmental conditions of a group of families in the arsenic-endemic blackfoot area of Taiwan. Fish was the only notable source of animal protein in most cases studied. The researchers concluded that, in general, the diet was adequate with respect to calories, high in carbohydrate, low in protein, and extremely low in fat. Undernourishment in the Taiwan population was marked by a high consumption of dried sweet potato, a staple food that was significantly associated with an increased prevalence of arsenic-induced skin cancer (Hsueh et al. 1995). In contrast, a larger variety of vegetables (although not fruits) were a part of the daily diet in West Bengal. However, a higher intake of fiber in controls than cases ($p = 0.02$) indicates a potato-based (low fiber content) diet for cases.

Low zinc intake was apparent in the study population, at least in comparison with the India RDAs. Engel and Receveur (1993) examined the full range of nutrients in the diet of the Taiwanese living in the blackfoot disease arsenic-affected area in the late 1950s. They calculated nutrient intake on the basis of the aggregated food consumption data presented by Yang and Blackwell (1961). The authors concluded that intakes of protein and essential amino acids were adequate, but participants had an average zinc intake of 9 mg/day, 60% of the recommended daily allowance of 15 mg/day (Engel and Receveur 1993). Zinc intake in our study population was similar to that reported in this study in Taiwan but did not differ between cases (average intake, 9.1 mg/day; 97.9% of participants below the RDA) and controls (average intake, 9.3 mg/day; 93.2% of participants below the RDA). However, it should be noted that various dietary recommendations have been made for zinc.

Skin lesions have also been reported in well-nourished populations. Smith et al. (2000) conducted a study in the village of Chiu Chiu in northern Chile. Family interviews identified a wide range of fruits and vegetables consumed daily by the affected participants, as well as the weekly intake of red meat and chicken. Although the study examined a small population, the prevalence of skin lesions among men and children was similar to the numbers reported in areas with corresponding arsenic concentrations in Taiwan and West Bengal, India (Guha Mazumder et al. 1998a; Smith et al. 2000). In two studies in West Bengal, India, there were mixed results with regard to use of chelating agents to enhance excretion of arsenic in patients with skin lesions, but there was overall improvement in skin lesions in patients both on the chelating agents and on the placebos (Guha Mazumder et al. 1998b, 2001). All patients were provided low-arsenic water and a hospital diet during the studies, so the role of nutrition in improvement, if any, could not be determined.

Conclusion

The findings of this study suggest that low vegetable fiber, low calcium, low folate, and low animal protein intake may play a role in increasing the risk of skin lesions in persons exposed to arsenic in drinking water. Our findings support an approximate doubling of risk for those with the lowest intake of these nutrients. Nevertheless, the potential protective effects associated with these nutrients were modest and require confirmation in other studies. We emphasize that the discontinuation of drinking arsenic-contaminated water and using "safe" drinking water remain of primary importance in managing patients with skin lesions.

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