Journal of Exposure Science and Environmental Epidemiology (2014) 24, 121–126

www.nature.com/jes

ORIGINAL ARTICLE

Arsenic exposure of rural populations from the Rift Valley of Ethiopia as monitored by keratin in toenails

R. Brittany Merola[1], Julia Kravchenko[2], Tewodros Rango[1] and Avner Vengosh[1]

Arsenic (As) contamination of drinking water is a worldwide phenomenon whose effect among vulnerable and rural communities in the Rift Valley of Ethiopia in eastern Africa is not well studied. This study examines As exposure and bioaccumulation from drinking water by monitoring human keratin in the form of toenails from exposed populations. Groundwater samples from drinking water wells ($n = 34$) were collected along with toenail samples ($n = 58$) from local communities and were analyzed for trace metals including As by inductively coupled plasma mass spectrometry (ICP-MS). Of the total number of wells tested, 53% had As level above the WHO maximum contamination level of 10 p.p.b. Arsenic in toenails was significantly correlated to corresponding drinking water ($r = 0.72$; $R^2 = 0.52$; $P < 0.001$). This correlation improves for drinking water with As concentrations above 2 p.p.b. ($r = 0.74$; $R^2 = 0.54$; $P < 0.001$). Male minors (<18 years old) were found to have greater nail–As concentrations compared with adults consuming equal amounts of As ($P < 0.05$). Estimated As dose specifically from drinking water sources was also associated with nail concentrations ($P < 0.01$). We suggest that As measurement in nails could be a reliable method for detecting As exposure in residents living in rural areas.

Journal of Exposure Science and Environmental Epidemiology (2014) **24,** 121–126; doi:10.1038/jes.2013.77; published online 6 November 2013

Keywords: arsenic; bioaccumulation; keratin; nails; Rift Valley; water quality

INTRODUCTION

Arsenic (As) exposure is a global phenomenon; millions consume drinking water with As concentration exceeding the WHO-recommended guideline of 10 p.p.b.[1] High levels of As exposure have been shown to cause acute health effects such as nausea, vomiting, abdominal pain, profuse diarrhea, renal failure, and shock,[2] whereas long-term exposure to relatively lower levels of As is known to increase the risk of chronic diseases such as lung, skin, kidney, urinary, bladder cancers, cardiovascular diseases, peripheral neuropathies, and diabetes, as well as many others.[3–12] Evaluating the symptoms and diseases directly associated with low-to-moderate As levels, yet still exceeding the maximum contaminant level (MCL) threshold (10 p.p.b.), is problematic because of the long latency period between chronic exposure and disease occurrence, many confounding factors, and multiple competitive risks (e.g., it has been shown that As can act as a synergistic carcinogen).[13–19] Understanding the correlation between moderate As exposure from the ingestion of drinking water and bioaccumulation in humans is challenging yet essential for evaluating health implications of long-term consumption of As-contaminated water.

Numerous studies have used keratin biomarkers in human subjects (e.g., hair and nails) for delineating the accumulation of As in the human body resulting from As exposure.[12,20–26] Here we present a unique case study in which the majority of participants living in the Rift Valley of Ethiopia only consume water from a single water source—a community well located in their village, which allows us to minimize error due to multiple drinking water sources. This study presents data of the relationships between As in drinking water and nails from rural communities in the Rift Valley of Ethiopia, where well water is the only available drinking water source and the participants report long residency times. We hypothesize that the magnitude of As exposure from drinking water for these populations can be reliably determined by nail–As analysis. In addition, basic demographic information, water consumption habits, and meat consumption patterns were collected and analyzed in order to understand the role they might have on As bioaccumulation in the residents.

Groundwater from the Ethiopian Rift Valley is known to contain high levels of fluoride, which is a serious health concern.[27–31] Yet, in addition to fluoride, recent studies have also documented As concentrations in drinking water above the WHO limit of 10 p.p.b.[28,32] Although fluoride may garner more attention because of its early and visible health effects, the potential effects of moderately elevated As ingestion are more insidious, causing long-term health effects that are difficult to correlate directly to water exposure. Evaluation of As exposure can be affected by multiple confounding factors that are not always visible during brief exams in the fields. In addition, other As exposure and health outcome monitoring factors such as smoking, sun exposure, nutrition, water consumption rates, age, gender, genetic factors, and water chemistry could affect the exposure evaluation.[12,23,33–38] Although there are some disagreements in the literature,[39] evidence points that malnutrition, particularly diets lacking animal protein, folic acid, calcium, vitamin A, and fiber are associated with increased incidences of As-induced skin lesion.[38,40] Nutrients such as animal proteins, folic acid, vitamin A, and fiber may therefore play a protective role in preventing negative health outcomes associated with As exposure by increasing the rate at which the methylation process can occur.[33,36,41]

[1]Division of Earth and Ocean Sciences, Nicholas School of the Environment, Duke University, Durham, North Carolina, USA and [2]Duke Clinical Research Institute, Duke University Medical Center, Durham, North Carolina, USA. Correspondence to: Professor Avner Vengosh, Division of Earth and Ocean Sciences, Nicholas School of the Environment, Duke University, 205 Old Chemistry Building, Box 90227, Durham, NC 27708, USA.
Tel.: +1 919 681 8050. Fax: +1 919 684 5833.
E-mail: vengosh@duke.edu
Received 27 December 2012; accepted 1 October 2013; published online 6 November 2013

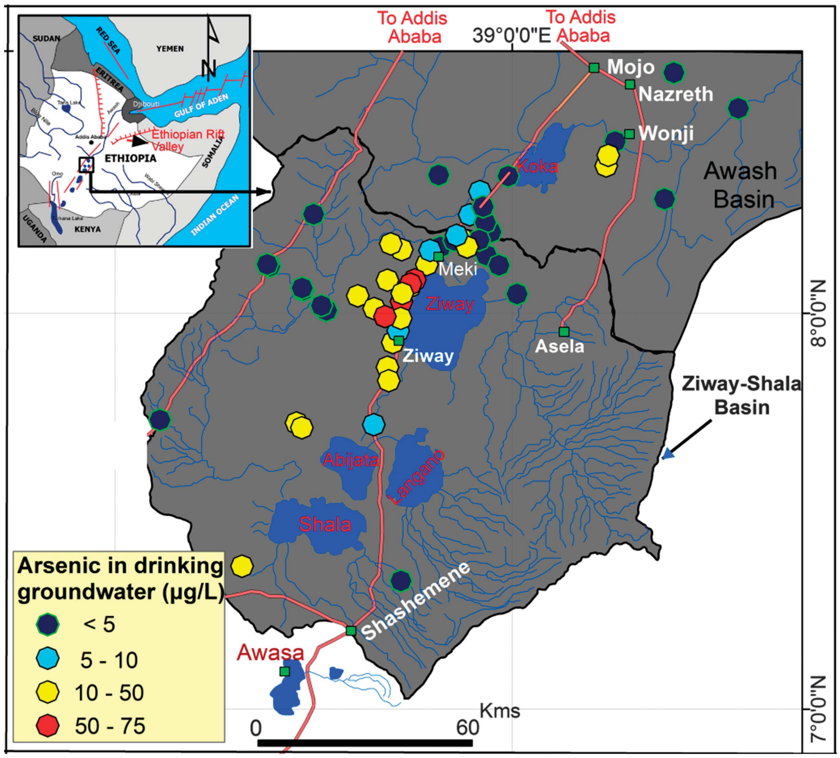

Figure 1. Location of drinking water wells in the Ethiopian Rift Valley and the spatial distribution of their arsenic (As) concentrations.

This study is focused on measuring As in nail samples of rural populations in the Ethiopian Rift Valley. Keratin, with its large quantity of the sulfhydryl-rich amino acid cysteine group, binds As and subsequently removes it from the metabolic process, preserving it in the nail material.[12,25] Nails in particular are thought to be a reliable biomarker because of their ease in collection and storage, and the lack of external contamination.[20,21,23,25] The slow growth of toenails represents an exposure window ranging from several months to a year and provides the base for understanding chronic accumulated exposures.[12,25,42] In addition, the slower growth of toenails compared with fingernails corresponds with a greater As content per equal mass of nail,[22,25,33] which makes its analysis more sensitive to changes in As in drinking water.

This study provides systematic measurements of As and its major inorganic species in drinking water wells combined with As measurements in toenails of residents who consume well water from selected villages in the Ethiopian Rift. The study aims to establish the relationship between As in water and nails for quantifying the magnitude of As bioaccumulation, understanding the factors that may modify exposure to As, and the role that As may be playing in the health of people in the region.

METHODS

Site Selection

The study area with a length of ~210 km is located in the main Ethiopian Rift Valley. In each town, local water officials were contacted and acted as guides to locate the drinking water wells. Wells were selected based on accessibility and whether or not they were functioning. As part of this study, 34 wells were tested. Figure 1 shows the location of these wells and their As concentrations.

At each location, researchers selected participants by intercepting local residents who were present at the well at the time of sampling. The survey questionnaire and study were conducted after ethical approval from the Duke University Institutional Review Board (IRB). An additional permission to carry out the project was also obtained from the Addis Ababa University and local institutes in the studied region (schools, water bureaus, and hospitals) after an explanation of the objectives and the method of study. Anonymity was also ensured to the investigated subjects.

Participants completed a questionnaire to document not only their general demographics such as age, gender, and residence time at the location, but also their water consumption habits, tobacco usage, general health status, and a basic nutrition assessment. Because all nail samples were collected within a 4-week period, seasonal variations in concentrations due to nail growth rate variations were not considered in our analysis.

Drinking Water Collection and Analysis

Well water samples were collected at the well site following US Geological Survey protocols along with pH, electrical conductivity, oxidation–reduction potential, dissolved oxygen, and temperature.[43] Samples were filtered at the well sites using 0.45 μm syringe filters and preserved using nitric acid. Samples were then shipped back to Duke University for analysis. More details of the analytical methodologies can be found in Ruhl et al.[44] Major elements were determined by direct current plasma optical emission spectrometry (DCP-OES) and anions by ion chromatography (IC). Trace elements were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Speciation of As was performed in the field using methods described in Bednar et al.[45] applying EDTA and anion exchange to separate the uncharged As(III) species and preserved separately in the field.

Nail Collection and Analysis

Toenails were collected from all 10 toes from participants using clean new clippers and stored in a Ziploc bag until the samples could be cleaned and analyzed. Visible dirt was removed by hand. Samples were cleaned with successive rinses of acetone, a 1% Triton X solution, and a second acetone rinse. Each rinse was sonicated for 30 min and followed with a sonicated water rinse for 30 min. After cleaning, the samples were baked overnight at 60 °C until totally dry. Samples were digested using ultra pure concentrated HNO_3 and H_2O_2 in a ratio of 1 ml HNO_3:100 μl H_2O_2:10 mg of nail using methods modified from Chen et al.,[46] Karagas et al.,[23] and Samanta et al.[24] After digestion, samples were sealed in Teflon containers and heated at a low temperature to ensure each sample's uniformity. An aliquot was then diluted with ultrapure water and run on the ICP-MS.

RESULTS

Arsenic was detected in all 34 selected wells, ranging from 0.6 to 73.4 p.p.b. with a mean of 18.6 p.p.b.; 19 out of 34 (53%) of the wells tested had As concentrations above the WHO limit of 10 p.p.b. The spatial distribution of As concentrations in well waters as well as the concentration frequencies are shown in Figures 1 and 2, respectively.

A total of 60 participants (43 males and 17 females) completed the study questionnaire, and 58 donated nail samples. The mean age of participants was 21 years, ranging from 8 to 58 years of age (male average age: 22; female average age: 21). The data show that As concentrations in nails were positively correlated with As concentrations in water ($r = 0.72$; $R^2 = 0.52$; $P < 0.001$). All participants who donated nails had lived in the area for at least 1 year (mean = 20 years, ranging from 4 to 57 years) and had been consuming water from the specific community well during that time period. The data were not normally distributed, and required log-transformation before statistical analysis.

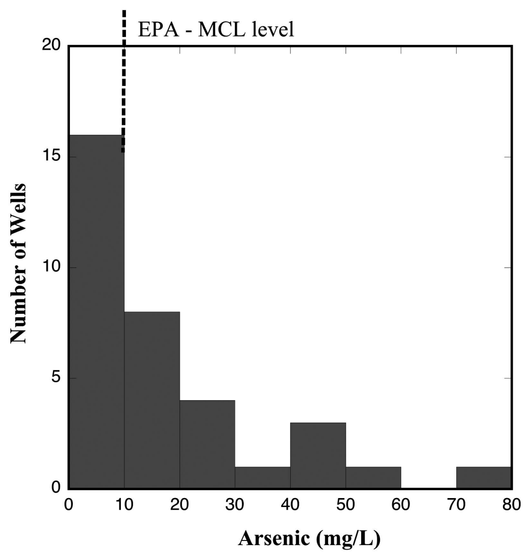

Figure 2. Histogram of arsenic (As) in drinking water wells tested. Out of 34 wells, 19 were above the WHO threshold drinking limit of 10 p.p.b.

The correlation between As–nail concentration and water concentration improved for water exposures above 2 p.p.b. (Figure 3a). The correlation obtained for nail–water pairs for population consuming drinking water with As > 2 p.p.b. was higher than the bulk data with an r value of 0.74 ($R^2 = 0.55$; $P < 0.001$).

The data show no statistically significant difference in nail concentrations between males and females, which may be a result of the limited number of females in the study. Upon arrival at each well location, researchers approached those present, explained the study, and asked for volunteers. Although males and females were both present at the wells, males were more likely to volunteer to participate, creating an inevitable male bias in the samples collected. There was a small but significant difference between adult males and minor males (defined as < 18 years of age; $P < 0.05$; Figure 3b). Minors were found to have greater As accumulation in their nails as compared with their adult counterparts, despite equal amounts of As in their drinking water, which has been reported in other studies investigating the correlation between As exposure and nail concentrations.[21]

In the oxidizing groundwater of the Ethiopian Rift valley,[28] the predominant As species is arsenate ($H_2AsO_4^-$, As(V)) relative to the reduced form arsenite (H_3AsO_3, As(III)). Arsenite is known to be more toxic to human health.[42,45,47] Our water quality data are consistent with Rango et al.[28,29,32] and show that the majority of the study wells were tested predominately comprised As(V); yet, wells with higher concentrations of As contained relatively more As(III) than those with lower As concentrations. Figure 4 shows the As speciation data from groundwater samples investigated in this study. Groundwater with As(V) comprising $> 90\%$ of the total As is shown with red circles, whereas groundwater with $< 90\%$ As(V) (i.e., higher As(III)) is shown with blue squares.

In addition to determining the relationship between As in water and nails, we evaluated the As dose for the participants by using the amount of water consumed by each individual and the As concentrations in the drinking water (Figure 5). Individuals were asked to self-report the amount of water they consumed. Dose was defined as $d = c \times v$, where d = dose, c = As concentration in water in parts per billion, and v = self-reported volume of water consumed per day in liters. Water consumption ranged from 0.5 l/day to 3 l/day, with a mean of 1.54 l/day, which is slightly below the WHO worldwide average of 2 l/day.[1] None of the participants in the study reported using bottled water, and 11 of

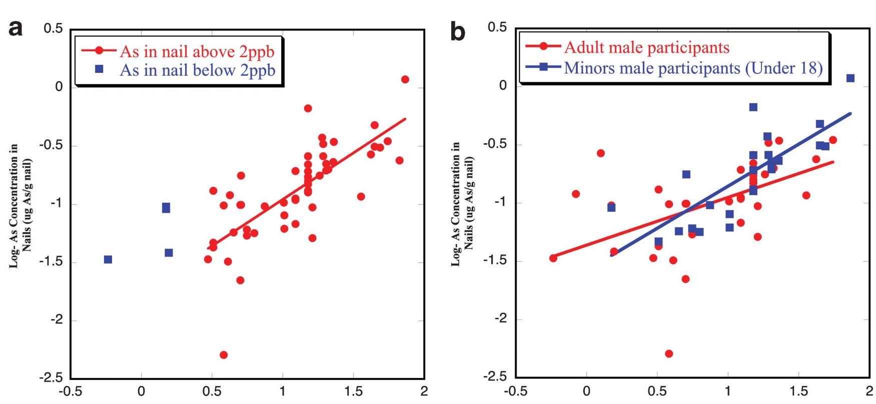

Figure 3. (a) Log-As concentration in nail *versus* log-As concentration in drinking water. For groundwater above 2 p.p.b. (red circles), higher correlation was obtained between arsenic (As) in nails and As in water ($r = 0.74$; $R^2 = 0.55$; $P < 0.001$) relative to groundwater below 2 p.p.b. (blue diamonds). (b) Log-As concentration in nail *versus* log-As concentration in drinking water for adult (> 18 years old; red circles; $r = 0.72$; $R^2 = 0.52$; $P < 0.001$) and minor males (blue squares; $r = 0.92$; $R^2 = 0.85$; $P < 0.001$). The two populations are significantly different from each other ($P < 0.05$).

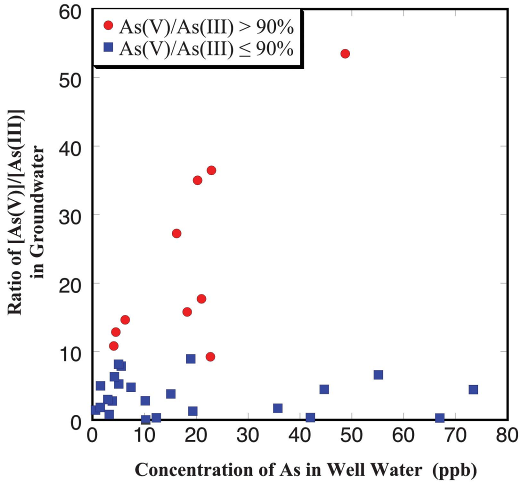

Figure 4. Ratios of As(V)/As(III) *versus* concentration of As in well water. Wells with predominance of As(V) that comprised at least 90% of the total dissolved arsenic (As) are marked by red circles relative to wells where As(V) was $<90\%$ (i.e., predominance of As (III)) marked as blue squares.

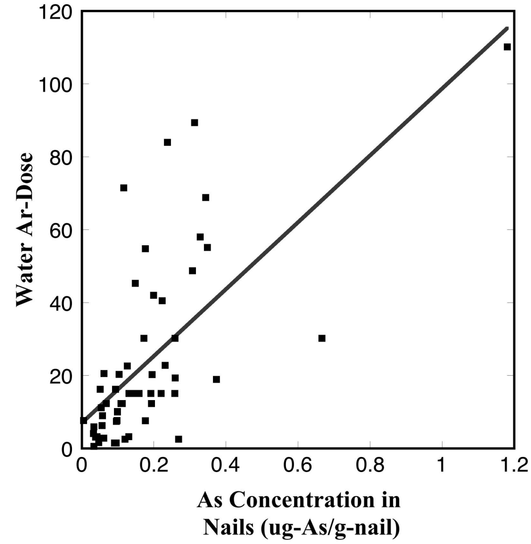

Figure 5. As–water dose (defined as arsenic (As) concentration in drinking water $\times$ self-reported volume of water consumed) *versus* As concentration in participants' nails. The data show a clear relationship between the amount of As consumed and As bioaccumulation ($r = 0.67$, $P < 0.01$).

the 59 participants reported rare usage of additional water sources (usually surface water sources) beside their well water source.

Basic nutrition information such as frequency of meat and milk consumed as well as general dietary patterns was collected as part of this study. Participants' diets consisted primarily of maize, injera (made of teff grain), and occasionally wheat. Because regular meat and dairy consumption was less common, a 24-h food recall survey would not have accurately reflected consumption of these foods, and therefore individual participants were asked their consumption patterns for these items explicitly. Mean meat intake for participants was 14.6 times/year (median 4 times/year). Dairy consumption was more common than meat consumption; participants reported consuming dairy $\sim$3 times/week on average (median 1.5 times/week). None of the participants reported any fish consumption, which could have potentially increased exposure to organic As present in fish.[34,48]

DISCUSSION

The As concentration in nail samples collected was significantly correlated with the As in participants' drinking water, showing that As is in fact bioaccumulating in people in this region consuming well water. It is important to note that bioaccumulation does not directly equate to negative health outcomes; however, it is a vital first step in assessing which populations should be targeted when evaluating health outcomes from As exposure.

Karagas et al.[23] found that there is a significantly improved correlation between nails and water when individuals are exposed to As in water concentrations above 1 p.p.b. This may be evidence for an exposure threshold at which the body begins to accumulate As that is reflected in the As level in the nails. Our data validate this trend, and the nail–water correlation improves for As water concentrations above 2 p.p.b. (Figure 3a). These data support the concept that the body burden of As shifts from background levels to accumulating the metalloid at a certain threshold level. The threshold our data show is slightly greater than that of Karagas et al.[23] (2 *versus* 1 p.b.b.); however, that is likely because of the low number of wells we measured with As near or below 1 p.p.b. Although it is not established that this threshold is directly linked to As health effects, it is important to note that As is already accumulating in the body below the water concentration that WHO considers safe, and health effects because of exposures in this range need to be investigated in the future.

One major limitation of this study was a lack of female participants. When researchers arrived at each well site, the study was explained to individuals present and the first few individuals to volunteer were recruited into the study. Although unintentional, males were more likely to volunteer. Males <18 years old were found to have greater As accumulation in their nails compared with their adult counterparts, a relationship that has been noted in previous studies.[20] Nail growth rate is maximized around ages 10–14 years.[51] Faster nail growth would result in lower As concentrations per equal mass of nail. In comparing As concentrations between adult males and minor males, we assume that nail growth is equal and therefore the rate of As deposited is the same in both subgroups. However, if children's nails grow faster than their adult counterparts, nails may actually be underestimating the As bioaccumulation in minors compared with adults. Minors (of both gender) in our study had higher concentrations in general than adults (of both gender); however, for females this difference was not statistically significant.

The As in the studied groundwater was overwhelmingly As(V) (as compared with As(III)). Although As(V) is thought to be slightly less toxic to humans than As(III), it is still harmful to consume.[42,45,47] In addition, wells with the highest levels of As had a relatively higher As(III) fraction (Figure 4). This means that individuals from the Rift Valley who are at a greater risk due to consuming larger concentrations of As are also at an increased health risk from consuming relatively greater amounts of As(III). Our data did not show any evidence for higher As in nails for individual consuming either As(III) or As (V); however, the small number of As–nail to predominantly As(III) pairs in our sampling pool makes this distinction difficult. Because of the lack of heterogeneity in As speciation in the water samples tested, the population in this study is not a good candidate for investigating the role that As speciation may play in As bioaccumulation in the nail.

Individuals were asked to self-report the amount of water they consumed in order to calculate a dose. Although recall error in the estimation of personal consumption is often a major source

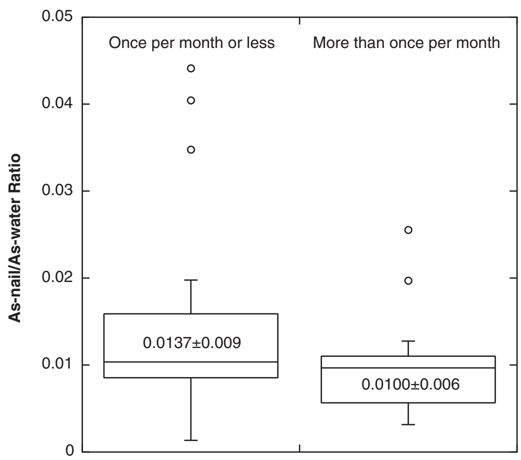

Figure 6. Boxplot showing that the ratios of arsenic (As) in nails to As in water differ in participants by their meat consumption patterns: meat consumption once per month or less ($n = 32$) *versus* meat consumption more than once per month ($n = 14$). This difference is statistically significant with the 80% CI, $P = 0.2$.

of bias, individuals in this region commonly used jerry cans and other water containers to transport water from the well to their home each day, and hence participants had a good understanding of how much water was brought into their home each day, and reported using only one water source. Our data suggest a clear relationship between dose of As from direct water intake and exposure. Although the average daily water intake for the study population was below the level at which the WHO bases their As limits in water, the data show that individuals consuming this amount are still being exposed at a non-negligible level, and that this exposure is reflected by As increasing in the nail even at concentrations of <10 p.p.b. in drinking water. Although not all exposure would occur from direct intake of As in water,[35] our data demonstrate that for the studied population, exposure from water alone correlates with As bioaccumulation. Consequently, this study shows that As intake from contaminated drinking water is playing a major role in As exposure.

The role of nutrition in health outcomes related to As exposure is an important research question that is still debated; although some studies question the protective role that nutrition may play in preventing negative health outcomes,[39] others have shown a decrease of skin lesions associated with better nutritional status, particularly with regard to calcium, animal protein, folate, and fiber intakes.[38,41] It has been proposed that the lack of animal protein in the diet may minimize the amount of glutathione (GSH) and other methyl groups in the body that play a key role in the excretion of As.[33,34,40,49,50] Although many studies have measured the concentrations of As compared with the incidence of health outcomes associated with As exposure, and even in some cases the concentrations of As in the food, no other studies to our knowledge have yet examined the relationships between biomarker As nail concentrations and nutrition. This association is an important step in understanding the relationship between bioaccumulation and nutrition.

Of the 55 participants, 36 (65%) consumed meat less than 5 times per year. Our data show that As–nail concentrations of individuals who consume meat once per month or less is higher compared with those who consume once per week or more for the same As concentrations in drinking water ($P < 0.2$; Figure 6). It should be noted that this difference was not significantly different at the $P < 0.05$ level, but it is significant with an 80% confidence interval. Given the variability in individual metabolisms, this difference is reasonable. These data variations are consistent with the observations that meat consumption may lower incidences of As-induced skin lesions.[33,34,39,49,50]

In conclusion, this study investigates the bioaccumulation of As in residents of rural communities of the Ethiopian Rift valley of Eastern Africa who are exposed to different levels of As in drinking water. Our study monitored large variations in As concentrations in well water (<1 to 70 p.p.b.). However, As bioaccumulation in the body, as revealed by As concentrations in nails of residents who consume well water, was also found in individuals who consume drinking water below the WHO guideline value of 10 p.p.b. A systematic correlation was found between As in drinking water and As in nails. We identified an empirical threshold of 2 p.p.b. from which As bioaccumulation strongly corresponds to As levels in drinking water. In addition, we show that poor nutrition and drinking water consumption habits also affect As bioaccumulation. This study found that As–nail analysis is an important and useful tool in assessing the body burden of As exposure, particularly for rural communities where health information is not available. Future work should assess the types of the specific health outcomes in populations who are exposed to such intermediate As levels in drinking water with measureable As bioaccumulation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Duke Global Health Institute, the Nicholas School of Environment, and the Problem-Focused Interdisciplinary Research-Scholarship Teams (PFIRST) program at Duke University for supporting the study. Special thanks are extended to the children, parents, school authorities, and water bureaus in the Rift Valley of Ethiopia for their kindly welcome and participation in the study. We thank Gary Dwyer, Laura Raul, Nathaniel Warner, and Tom Darrah from the Nicholas School of Environment of Duke University for laboratory analytical assistance. We also thank two anonymous reviewers for their comments.

REFERENCES

1 World Health Organization. *Guidelines for Drinking Water Quality*. 4th edn. WHO Press: Geneva, Switzerland, 2011.
2 Huang YZ, Qian XC, Wang GQ, Wiao BY, Ren DD, Feng ZY *et al.* Endemic chronic arsenism in Xinjiang. *Clin Med J* 1985; **98**: 219–222.
3 Abernathy C, Liu YP, Longfellow D, Aposhian V, Beck B, Fowler B *et al.* Arsenic: health effects, mechanisms of actions and research issues. *Environ Health Perspect* 1999; **107**: 593–597.
4 Chen CJ, Chen CW, Wu MM, Kuo TL. Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. *Br J Cancer* 1992; **66**: 888–892.
5 Chen CJ, Hsu LI, Wang CH, Shih WL, Hsu YH, Tseng MP *et al.* Biomarkers of exposure, effect, and susceptibility of arsenic-induced health hazards in Taiwan. *Toxicol Appl Pharmacol* 2005; **206**: 198–206.
6 Chouhan S, Flora SJS. Arsenic and fluoride: two major ground water pollutants. *Indian J Exp Biol* 2010; **48**: 666–678.
7 Kapaj S, Peterson H, Liber K, Bhattacharya P. Human health effects from chronic arsenic poisoning—a review. *J Environ Sci Health Part A Tox Hazard Subst Environ Eng* 2006; **41**: 2399–2428.
8 Kitchin KT. Recent advances in arsenic carcinogenesis: models of action, animal model systems, and methylated arsenic metabolite. *Toxicol Appl Pharmacol* 2001; **172**: 249–261.
9 Meliker JR, Wahl RL, Cameron LL, Nriagu JO. Arsenic in drinking water and cerebrovascular disease, diabetes mellitus and kidney disease in Michigan: a standardized mortality ratio analysis. *Environ Health* 2007; **6**: 4.
10 Morales KN, Ryan L, Kuo TL, Wu MM, Chen CJ. Risk of internal cancers from arsenic in drinking water. *Environ Health Perspect* 2000; **108**: 655–661.
11 National Research Council. Arsenic in Drinking Water: 2001 Update. *Subcommittee on Arsenic in Drinking Water*. National Academy of Sciences: Washington, DC, 2001.
12 Yoshida T, Yamauchi H, Sun GF. Chronic health effects in people exposed to arsenic via the drinking water: dose-response relationships in review. *Toxicol Appl Pharmacol* 2004; **198**: 243–252.

13 Bates MN, Smith AH, Cantor KP. Case-control study of bladder cancer and arsenic in drinking water. *Am J Epidemiol* 1995; **141**: 523–530.
14 Bates MN, Rey OA, Biggs ML, Hopenhayn C, Moore LE, Kalman D *et al.* Case-control study of bladder cancer and exposure to arsenic in Argentina. *Am J Epidemiol* 2004; **159**: 381–389.
15 Chen CL, Hsu LI, Chiou HY, Hsueh YM, Chen SY, Wu MM *et al.* Ingested arsenic, cigarette smoking, and lung cancer risk: a follow-up study in arseniasis-endemic areas in Taiwan. *JAMA* 2004; **292**: 2984–2999.
16 Chen Y, Graziano JH, Parvez F, Hussain I, Momotaj H, van Geen A. Modification of risk of arsenic-induced skin lesions by sunlight exposure, smoking, and occupational exposure in Bangladesh. *Epidemiology* 2006; **17**: 459–467.
17 Ferreccio C, González C, Milosavjlevic V, Marshall G, Sancha AM, Smith AH. Lung cancer and arsenic concentrations in drinking water in Chile. *Epidemiology* 2000; **11**: 673–679.
18 Lindberg AL, Sohel N, Rahman M, Persson LA, Vahter M. Impact of smoking and chewing tobacco on arsenic-induced skin lesions. *Environ Health Perspect* 2010; **118**: 533–538.
19 Ratnaike RN. Acute and chronic arsenic toxicity. *Postgrad Med J* 2003; **79**: 391–396.
20 Garland M, Morris JS, Rosner BA, Stampfer MJ, Spate VL, Baskett CJ *et al.* Toenail trace element levels as biomarkers: reproducibility over a 6-year period. *Cancer Epidemiol Biomarkers Prev* 1993; **2**: 493–497.
21 Hinwood AL, Sim MR, Jolley D, de Klerk N, Bastone EB, Gerostamoulos J *et al.* Hair and toenail arsenic concentrations of residents living in areas with high environmental arsenic concentrations. *Environ Health Perspect* 2003; **111**: 187–193.
22 Karagas MR, Morris JS, Weiss JE, Spate V, Baskett C, Greenberg ER. Toenail samples as an indicator of drinking water arsenic exposure. *Cancer Epidemiol Biomarkers Prev* 1996; **5**: 849–852.
23 Karagas MR, Tosteson TD, Blum J, Klaue B, Weiss J, Stannard V *et al.* Measurement on low levels of arsenic exposure: a comparison of water and toenail concentrations. *Am J Epidemiol* 2000; **152**: 84–90.
24 Samanta G, Sharma R, Roychowdhury T, Chakraborti D. Arsenic and other elements in hair, nails, and skin-scales of arsenic victims in West Bengal, India. *Sci Total Environ* 2004; **326**: 33–47.
25 Slotnick MJ, Nriagu JO. Validity of human nails as a biomarker of arsenic and selenium exposure: a review. *Environ Res* 2006; **102**: 125–139.
26 Pearce D, Dowling K, Gerson A, Sim M, Sutton S, Newville M *et al.* Arsenic microdistribution and speciation in toenail clippings of children living in a historic gold mining area. *Sci Tot Environ* 2010; **408**: 2590–2599.
27 Gizaw B. The origin of high bicarbonate and fluoride concentrations in waters of the Main Ethiopian Rift Valley, East African Rift System. *J Afr Earth Sci* 1996; **22**: 391–402.
28 Rango T, Bianchini G, Beccaluva L, Tassinari R. Geochemistry and water quality assessment of central Main Ethiopian Rift natural waters with emphasis on source and occurrence of fluoride and arsenic. *J Afr Earth Sci* 2010; **57**: 479–491.
29 Rango T, Kravchenko J, Atlaw B, McCornick PG, Jeuland M, Merola B *et al.* Groundwater quality and its health impact: an assessment of dental fluorosis in rural inhabitants of the Main Ethiopian Rift. *Environ Int* 2012; **43**: 37–47.
30 Tekle-Haimanot R, Melaku Z, Kloos H, Reimann C, Wondwossen F, Zerihun L *et al.* The geographic distribution of fluoride in surface and groundwater in Ethiopia with an emphasis on the Rift Valley. *Sci Total Environ* 2006; **367**: 182–190.
31 Reimann C, Bjorvatn K, Frengstad B, Melaku Z, Tekle-Haimanot R, Siewers U. Drinking water quality in the Ethiopian section of the East African Rift Valley I-data and health aspects. *Sci Total Environ* 2003; **311**: 65–80.
32 Godebo TR, Vengosh A, Dwyer G, Bianchini G. Mobilization of arsenic and other naturally occurring contaminants in groundwater of the Main Ethiopian Rift aquifers. *Water Res* 2013; **47**: 5801–5818.
33 Brima EI, Haris PI, Jenkins RO, Polya DA, Gault AG, Harrington CF. Understanding arsenic metabolism through a comparative study of arsenic levels in the urine, hair and fingernails of health volunteers from three unexposed ethnic groups in the United Kingdom. *Toxicol Appl Pharmacol* 2006; **216**: 122–130.
34 Gebel T. Confounding variables in the environmental toxicology of arsenic. *Toxicology* 2000; **144**: 155–162.
35 Hossain MA, Rahman MM, Murrill M, Das B, Roy B, Dey S *et al.* Water consumption patterns and factors contributing to water consumption in arsenic affected populations of rural West Bengal, India. *Sci Total Environ* 2012; **463-464**: 1217–1224.
36 Pierce BL, Argos M, Chen Y, Melkonian S, Parvez F, Islam T *et al.* Arsenic exposure, dietary patterns, and skin lesion risk in Bangladesh: a prospective study. *Am J Epidemiol* 2011; **173**: 345–354.
37 Vahter M, Berglund M, Åkesson A, Liden C. Metals and women's health. *Environ Res Section A* 2002; **88**: 145–155.
38 Zablotska LB, Chen Y, Graziano JH, Parvez F, van Geen A, Howe GR *et al.* Protective effects of B vitamins and antioxidants on the risk of arsenic-related skin lesions in Bangladesh. *Environ Health Perspect* 2008; **116**: 1056–1062.
39 Smith AH, Arroyo AP, Mazumder DNG, Kosnett MJ, Hernandez AL, Beeris M *et al.* Arsenic-induced skin lesions among Atacameño people in Northern Chile despite good nutrition and centuries of exposure. *Environ Health Perspect* 2000; **108**: 617–620.
40 Anetor JI, Wanibuchi H, Fukushima S. Arsenic exposure and its health effects and risk of cancer in developing countries: micronutrients as host defence. *Asian Pac J Cancer Prev* 2007; **8**: 13–23.
41 Mitra SR, Mazumder DN, Basu A, Block A, Haque R, Samanta S *et al.* Nutritional factors and susceptibility to arsenic-caused skin lesions in West Bengal, India. *Environ Health Perspect* 2004; **112**: 1104–1109.
42 Schroeder HA, Balassa JJ. Abnormal trace metals in man: arsenic. *J Chron Dis* 1966; **19**: 85–106.
43 US Geological Survey. *National field manual for the collection of water quality data*. US Geological Survey: Washington, DC, 2011.
44 Ruhl L, Vengosh A, Dwyer GS, Hsu-Kim H, Deonarine A. Environmental impacts of the coal ash spill in Kingston, Tennessee: an 18-month survey. *Environ Sci Technol* 2010; **44**: 9272–9278.
45 Bednar AJ, Garbarino JR, Ranville JF, Wildeman TR. Preserving the distribution of inorganic arsenic species in groundwater and acid mine drainage samples. *Environ Sci Technol* 2002; **36**: 2213–2218.
46 Chen KLB, Amarasiriwardena CH, Christiani DC. Determination of total arsenic concentrations in nails by inductively coupled plasma mass spectrometry. *Biol Trace Elem Res* 1999; **67**: 109–125.
47 Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM *et al.* Cancer risks from arsenic in drinking water. *Environ Health Perspect* 1992; **97**: 259–267.
48 Liao CM, Shen HH, Lin TL, Chen SC, Chen CL, Hsu LI *et al.* Arsenic cancer risk posed to human health from tilapia consumption in Taiwan. *Ecotoxicol Environ Saf* 2008; **70**: 27–37.
49 Styblo M, Serves SB, Cullen WR, Thomas DJ. Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. *Chem Res Toxicol* 1997; **10**: 27–33.
50 Vahter M. Mechanism of arsenic biotransformation. *Toxicology* 2002; **181-182**: 211–217.
51 Dawber RPR, de Berker DAR, Baran R. The science of the nail apparatus. In: Baran R, Dawber RPR, de Berker DAR, Haneke E, Tosti A (eds). *Diseases of the Nails and their Management*. Blackwell Science: Malden, MA, USA, 2001, pp 1–47.