Arsenic exposure from drinking water and endothelial dysfunction in Bangladeshi adolescents

Shohreh F. Farzan[1,*], **HEM Mahbubul Eunus**[2], **Syed Emdadul Haque**[2], **Golam Sarwar**[2], **AKM Rabiul Hasan**[2], **Fen Wu**[3], **Tariqul Islam**[2], **Alauddin Ahmed**[2], **Mohammad Shahriar**[2,4], **Farzana Jasmine**[4], **Muhammad G. Kibriya**[4], **Faruque Parvez**[5], **Margaret R. Karagas**[6], **Yu Chen**[3], **Habibul Ahsan**[4]

[1]Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA

[2]UChicago Research Bangladesh, Dhaka, Bangladesh

[3]Department of Population Health, New York University, New York, NY

[4]Department of Public Health Sciences, University of Chicago, Chicago, IL,

[5]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY

[6]Department of Epidemiology, Geisel School of Medicine at Dartmouth, Hanover, NH

Abstract

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide, with ~80% of CVD-related deaths occurring in low- and middle-income countries. Growing evidence suggests that chronic arsenic exposure may contribute to CVD through its effect on endothelial function in adults. However, few studies have examined the influence of arsenic exposure on cardiovascular health in children and adolescents. To examine arsenic's relation to preclinical markers of endothelial dysfunction, we enrolled 200 adolescent children (ages 15–19 years; median 17) of adult participants in the Health Effects of Arsenic Longitudinal Study (HEALS), in Araihazar, Bangladesh. Participants' arsenic exposure was determined by recall of lifetime well usage for drinking water. As part of HEALS, wells were color-coded to indicate arsenic level (<10 μg/L, 10–50 μg/L, >50 μg/L). Endothelial function was measured by recording fingertip arterial pulsatile volume change and reactive hyperemia index (RHI) score, an independent CVD risk factor, was calculated from these measurements. In linear regression models adjusted for participant's sex, age, education, maternal education, land ownership and body weight, individuals

---

[*]**Corresponding author**: Shohreh F. Farzan, Department of Preventive Medicine, Division of Environmental Health, 2001 N Soto Street, Los Angeles, CA 90032. sffarzan@usc.edu; Telephone: 323-442-5101.

who reported always drinking water from wells with >50 μg/L arsenic had a 11.75% lower level of RHI (95% CI: −21.26, −1.09, p=0.03), as compared to participants who drank exclusively from wells with 50 μg/L arsenic. Sex-stratified analyses suggest that these associations were stronger in female participants. As compared to individuals who drank exclusively from wells with 50 μg/L arsenic, the use of wells with >50 μg/L arsenic was associated with 14.36% lower RHI (95% CI: −25.69, −1.29, p=0.03) in females, as compared to 5.35% lower RHI (95% CI: −22.28, 15.37, p=0.58) in males for the same comparison. Our results suggest that chronic arsenic exposure may be related to endothelial dysfunction in adolescents, especially among females. Further work is needed to confirm these findings and examine whether these changes may increase risk of later adverse cardiovascular health events.

Introduction

The World Health Organization estimates that more than 200 million people may be chronically exposed to inorganic arsenic (As) contaminated groundwater, exceeding the WHO standard of 10μg/L, with areas of concern documented worldwide, including Bangladesh, Chile, Taiwan, and the United States.[1–3] Arsenic contamination of drinking water in these areas results largely from natural geological deposits and anthropogenic sources.[1, 2, 4] Among highly exposed populations, arsenic has been linked to significant health effects including skin and internal cancers, diabetes, adverse pregnancy outcomes, immune suppression and neurological effects.[2, 5] Chronic arsenic exposure, across a range of exposure levels, has also been implicated in the development of cardiovascular disease (CVD). As CVD remains the leading cause of death worldwide, and continues to rise, with nearly 80% of CVD deaths occurring in low- and middle-income countries, understanding the contribution of environmental factors, such as arsenic, to the development of CVD is critical.[6] In 2013, a National Research Council committee on arsenic toxicity stated in their report that "CVD may be the most important non-cancer disease risk posed by environmental arsenic exposure".[7] Indeed, there is a growing body of evidence that arsenic exposure can significantly impact risk of CVD, including studies that have reported associations between arsenic exposure and CVD mortality, peripheral vascular disease, ischemic heart disease, and hypertension.[8–18] While evidence of arsenic's effects on clinical CVD outcomes has been limited to adults, studies among children have reported arsenic-related subclinical cardiovascular effects, including increased blood pressure, carotid intima media thickness, oxidative stress and echocardiographic parameters.[19–22]

Arsenic is thought to promote CVD pathogenesis through several mechanisms, including increased inflammation, endothelial damage and vascular lesion development.[23–25] Animal models echo these findings and have linked arsenic exposure to endothelial dysfunction, vascular remodeling, and increased angiogenesis.[26–29] Adverse effects of arsenic in vascular smooth muscle and endothelial cells have been observed at doses much lower than are required to induce cancer[26, 30], suggesting that endothelial cells may be a more sensitive target for arsenic. The endothelium is a key regulator of vascular homeostasis, and in adults, endothelial dysfunction is an independent risk factor for CVD and early detectable stage of cardiovascular pathogenesis among adults, often preceding the development of atherosclerosis.[31, 32] Endothelial dysfunction is often characterized by

abnormal vasoreactivity, which has been related to increased cardiovascular risk and may be attributable to damage to the endothelium, reduced availability of nitric oxide or an imbalance in endothelium-related relaxing and contracting factors.[32] While arsenic's effects on endothelial cells and vascular function have been investigated using endothelial-related biomarkers in several studies among adults[23–25, 33], as well as children[20, 34], to our knowledge, only one prior study has investigated arsenic's relationship to functional measure of endothelial responsiveness.[35] Functional measurements, including flow-mediated dilation and pulse amplitude tonometry, which allow for calculation of a reactive hyperemia index score, are a direct measure of vascular responsiveness and the dynamic biology of the endothelium, and therefore may be more sensitive than biomarker measurements.[32] In younger populations of children and adolescents, such measures may represent an important early indicator of adverse vascular changes.[36, 37]

We hypothesized that arsenic exposure would be associated with adverse changes in endothelial function in adolescence. In this study, we examined the association between lifetime well water arsenic levels and reactive hyperemia index, measured non-invasively by fingertip pulse amplitude tonometry, in 200 Bangladeshi adolescents.

Methods

Study population

Between January and December 2019, we enrolled 200 adolescents (ages 15–19 years; median: 17 years), identified as children of female adult participants in the Health Effects of Arsenic Longitudinal Study (HEALS), an established population-based study in rural Bangladesh (Araihazar) with varying levels and patterns of arsenic exposure.[38] These adolescents were invited to participate through random selection of 430 individuals from a pool of 900 adolescents between the ages of 14–19 years who were previously identified as eligible by village health workers as part of bi-annual HEALS follow-up visits, which documented the number of children and recent pregnancies among HEALS participants. Based on mothers' well arsenic level, we previously defined four groups with varying levels and patterns of exposure to arsenic among these adolescents: 1) consistently low; 2) consistently moderate; 3) consistently high; and 4) high from conception through roughly age one, then much lower.[38] Of the 430 individuals contacted, 370 agreed to participate in an initial screening questionnaire, and 310 completed it. Of these, 200 were enrolled and underwent a clinical evaluation, laboratory-based assays, and provided additional information by questionnaire surveys. Thus, the current analysis includes 200 adolescents with past well use history, endothelial function measurements, and complete covariate data. No evidence of selection bias was observed, as distributions of demographics and arsenic exposure in the 430 randomly selected participants were similar to those in the final sample of 200 (data not shown). The research protocol was approved by the University of Southern California Institutional Review Board (HS-16–00462) and the Bangladesh Medical Research Council (BMRC/NREC/2016–2019/930).

Water Arsenic Measurement and Exposure Assignment

Participant's water arsenic exposure was determined by recall of lifetime well usage. Wells in the study area are color coded to indicate their arsenic level (blue <10 μg/L, green 10–50 μg/L, or red >50 μg/L arsenic).[39] Participants were asked to recall the color of their current well and any past wells used, including number of years drinking from each well. Participants' mothers were invited to visit the healthcare center with their children during the assessment and approximately 80% of participants attended their clinical evaluation with their mother. When a participant's mother was present, she was asked to assist with well recall questions, as recalling wells from early life may be difficult if a family has not lived in the same residence and/or changed residences in early childhood. Therefore, the majority of participants either drank from the same well their entire lives (n=137) and/or had their mother present to assist with recall. If well color could not be recalled for a time period, most proximally recalled well color was assigned for the unknown period. For example, if current well color could not be recalled, current well was assigned the most recently recalled well color. Of the 200 participants, 18% (n=36) participants could not recall current well.

Lifetime exposure levels were estimated by grouping individuals by reported well usage. Individuals who reported either always drinking from blue wells (<10 μg/L) or a combination of blue and green wells (10–50 μg/L) were considered to have lower exposure and were assigned as the reference group for analyses. Individuals who reported always drinking from green wells (10–50 μg/L) or ever drank from a red well (>50 μg/L) were considered to have moderate exposure. Individuals who drank exclusively from red wells (>50 μg/L arsenic) were considered to have the highest lifetime exposure. The majority of participants were able to provide complete information about lifetime well use (n=135). Of the 65 (32.5%) participants who had some missingness in their recall of lifetime well use, the average gap in recall was 4.9 years, with the majority of participants (n=40) having 5 or less years of unknown well color. To validate participant well recall and exposure assignments, we linked maternal baseline well arsenic information, measured as part of the parent HEALS study protocol. Of the 200 participants in our study, 79 had maternal water arsenic information obtained within ~2 years of their birth, which was subsequently matched to participants first reported well color (Table S1).

Assessment of covariates

Individual sociodemographic and household characteristics were obtained from a self-reported questionnaire obtained upon enrollment. Variables included years of formal education, current school attendance, current smoking status, and smokers within the home. Adolescents' characteristics, including age and sex were also recorded at the time of the exam. Maternal characteristics, including education and land ownership, evaluated as part of prior assessments of HEALS participants were linked to adolescent participants. At the study clinic, the participant's height, weight and blood pressure were measured. Participants were asked to rest for 5 minutes prior to blood pressure measurement, which consisted of two measurements obtained in succession at 1-minute intervals with an automatic oscillometric device (Omron Healthcare GmbH, Hamburg, Germany). The two readings were averaged to provide mean systolic and average diastolic blood pressure measurements.

In-person assessment and endothelial function measurement

Endothelial function was measured by recording fingertip arterial pulsatile volume change using Endo-PAT2000 (Itamar Medical). Participants were asked to refrain from eating or drinking anything except water in the three hours prior to testing. Participants were given at least 20 minutes prior to testing to acclimate to the inside temperature, while being prepared for EndoPAT testing. The clinic room was kept dark and quiet to reduce environmental stimuli during testing and participants were asked to rest in a supine position and remain quiet for the duration of the testing. Briefly, the computer-interfaced Endo-PAT2000 automated instrument was attached to the participant through two fingertip probes, which detect arterial pulsatile blood-volume changes in the microvasculature, while the participant is at rest. The measurement includes an initial 6-minute baseline reading pre-occlusion, a 5-minute cuff-mediated occlusion of the brachial artery in the non-dominant arm, followed by 6 minutes of post-occlusion reading to determine the patient's reactive hyperemia index (RHI) score (i.e. the change in vascular tone post-occlusion), which is calculated on a continuous scale, using the non-occluded arm as a control signal. RHI and ln-RHI were calculated by the Itamar proprietary software. Lower values of RHI and ln-RHI are indicative of reduced endothelial function and have been correlated with endothelial dysfunction as measured by brachial ultrasound assessment of flow mediated dilation.[40–42] Lower RHI has also been associated with traditional cardiovascular risk factors in the Framingham Heart Study.[43]

Statistical analysis

All analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, NC). Complete exposure and RHI data were available for all 200 participants. Descriptive characteristics were calculated for all sociodemographic and individual characteristic variables. Mean and standard deviation were calculated for continuous variables and distribution (N, %) for categorical variables. We examined distributions of reactive hyperemia index (RHI). Given the skewed distribution of RHI, natural-log transformed reactive hyperemia index (lnRHI) was selected as our primary outcome variable and analyzed continuously.

First, we examined univariate associations of sociodemographic and individual characteristic variables with ln-RHI using linear regression models (Table S2). From these analyses, we selected potential confounding variables to include in adjusted linear regression models. Final models were adjusted for participant sex, age, education level and weight at the time of RHI measurement. To further adjust for socioeconomic factors, we additionally included maternal education and land ownership. We also tested the effect of including other covariates in models and additional adjustment for other potential confounders (e.g. SBP, any smoker at home) generated similar results (data not shown). To aid interpretation of our results, effect estimates of RHI and associated confidence intervals were back-transformed by exponentiating and subtracted by 1, and then multiplied by 100 for easier interpretation of the results as percent differences in RHI. P-values for individual groups are shown and p-trend values were calculated using dummy variables representing levels of current or lifetime exposure.

Given that less is known about possible differences in endothelial function by sex, sex-specific associations with lnRHI were evaluated for both lifetime well usage and current well usage. Stratified models were used to examine the strata-specific estimates and 95% confidence intervals. We also conducted sensitivity analyses to explore the influence of the well categorization method and assignment of unknown values on the observed associations. First, we examined the influence of unknown well color on the observed effects by analyzing "unknown current well" separately, rather than assigning the last recalled well color to individuals with an unknown current well. We also explored whether further separating individuals who were considered to have moderate exposure (either always drinking from green wells (<10 μg/L) or ever drank from a red well (>50 μg/L)) into two categories would influence observed associations. We examined the impact of imputing lifetime well usage for those who could not recall past well usage for 10 or more years (n=7) by excluding these individuals. Lastly, we explored whether we were able to observe similar associations among individuals who reported drinking from the same well over their entire lifetime (n=137), in an effort to reduce potential error introduced by inability to recall early life wells.

Results

Table 1 shows characteristics of the participants, both for the overall study population and categorized by lifetime well arsenic exposure. Slightly more than half of participants were male (53%) and on average, participants were 17.4 (SD: 0.9) years of age. The majority (70.5%) were currently attending school and more than a third (38.5%) had a higher secondary education or greater. Only 2% reported currently smoking, but half (50.5%) reported living with a smoker. On average, participants were 159.0 (SD: 9.5) cm tall and weighed 50.3 (SD: 9.0) kg. Mean systolic blood pressure was 111.1 (SD: 11.5) mmHg, with a wide range across participants (86.5–146.5 mmHg). Mean diastolic blood pressure was 68.2 (SD: 7.0) and ranged from 50.0–86.0 mmHg. Mean RHI determined by EndoPAT was 1.52 (SD: 0.43) and ranged from 0.78–3.28. Current well color varied among participants, with 15% reported currently using a blue well (<10 μg/L), 30.5% reported currently using a green well (10–50 μg/L) and more than a third (36.5%) reported using a red well (>50 μg/L). The remaining 18% could not recall the color of their current well. For 79 (39.5%) of the participants in our study, we were able to match participants first reported well color to information on their mother's well arsenic level obtained within ~2 years of their birth (Table S1). Overall, mean levels of measured maternal well arsenic was lowest (28.5 μg/L; SD: 28.4) among participants who reported using a blue well (<10 μg/L) as their first well, and greatest (74.3 μg/L; SD: 76.7) among those who reported using a red well (>50 μg/L) as their first well.

First, we assessed the association between lifetime well arsenic level and percent difference in endothelial function, shown in Table 2. In linear regression models adjusted for participant sex, age, education and weight at the time of RHI measurement and maternal education and land ownership, individuals who reported always using wells with >50 μg/L arsenic had a lower level of RHI by 11.75% (95% CI: −21,26, −1.09, p=0.03), as compared to participants who drank exclusively from wells with 50 μg/L arsenic (i.e. always blue or blue and green). Individuals with moderate exposures (i.e. always green or ever red) also had

a lower level of RHI by 7.69% (95% CI: −17.30, 3.25, p=0.16), as compared to participants who drank exclusively from wells with 50 μg/L arsenic (i.e. always blue or blue and green), but did not reach statistical significance. Comparison across categories of increasing lifetime well arsenic exposure was statistically significant (p-trend= 0.03).

We then assessed the association between current well arsenic level and percent difference in endothelial function, shown in Table 3. In linear regression models adjusted for participant sex, age, education and weight at the time of RHI measurement and maternal education and land ownership, we observed lower levels of RHI in individuals who reported currently using wells with >50 μg/L arsenic (−7.32%, 95% CI: −16.18, 2.43, p=0.14), and in those who reported currently using wells with 10–50 μg/L arsenic (−3.82%, 95% CI: −13.30, 6.66, p=0.45), as compared to participants who currently drank from wells with <10 μg/L. However, these differences did not reach statistical significance, nor did the overall trend (p-trend=0.13).

We also investigated sex-specific differences in associations between lifetime well arsenic level and percent difference in endothelial function, shown in Table 4. We observed greater reduction in RHI across arsenic exposure categories among female participants. Female participants who reported always using wells with >50 μg/L arsenic had a 14.36% lower RHI (95% CI: −25.69, −1.29, p=0.03), as compared to 5.35% lower RHI (95% CI: −22.28, 15.37, p=0.58) in males for the same comparison. Female participants with moderate exposures (i.e. always green or ever red) also had a lower level of RHI by 10.33% (95% CI: −21.96, 3.01, p=0.12), as compared to participants who drank exclusively from wells with 50 μg/L arsenic (i.e. always blue or blue and green), but did not reach statistical significance. Comparison across categories of increasing lifetime well arsenic exposure among female participants was statistically significant (p-trend= 0.04). However, we did not observe an interaction for sex and lifetime well exposure (p-interaction= 0.57). Similar trends were observed in sex-stratified analyses of current well color and RHI, but these results did not reach statistical significance (Table S3).

In a series of sensitivity analyses, we explored the influence of our well categorization scheme on the observed associations. First we examined the influence of assigning the last recalled well color to individuals with an unknown current well on the effect estimates. We observed similar estimates among those in the highest exposure category (>50 μg/L arsenic) with an 8.61% lower RHI (95% CI: −18.13, 2.20, p=0.13). Those with unknown current well also had a 7.69% lower RHI (95% CI: −18.94, 4.08, p=0.20). However, none of these estimates reached statistical significance (Table S4). We then examined the influence of those who were missing 10 or more years of well color recall by excluding these 7 individuals. Exclusion of those with more than 10 years of missingness in well recall did not greatly influence our results and similar estimates were observed (Table S5). We then explored whether further separating individuals who were considered to have moderate exposure (either always drinking from green wells (<10 μg/L) or ever drank from a red well (>50 μg/L)) into two categories would influence observed associations and found that the overall trends remained similar (Table S6). Individuals who reported always drinking from a green well (10–50 μg/L arsenic) had a lower level of RHI by 10.41% (95% CI: −20.55, 2.02, p=0.10), as compared to participants who drank exclusively from wells

with ≤50 μg/L arsenic (i.e. always blue or blue and green), but did not reach statistical significance. Individuals who reported ever using red wells with >50 μg/L arsenic had a lower level of RHI by −5.82% (95% CI: −16.47, 6.18, p=0.32), as compared to participants who drank exclusively from wells with ≤50 μg/L arsenic (i.e. always blue or blue and green), but this trend was not statistically significant. Estimates among individuals with who drank exclusively from wells with >50 μg/L arsenic remained unchanged and statistically significant (p=0.03). When these separate categories of exposure were used in stratified models, the effects among female participants remained very similar (Table S7). When we restricted our analyses to only participants who reported that they drank from a single well for their entire lifetime, in order to reduce potential error introduced by inability to recall early life wells, we observed similar reductions in RHI among those who always drank from green or red wells, as compared to those who always drank from blue wells (Table S8). However, these results did not reach statistical significance.

Discussion

In our analysis of 200 Bangladeshi adolescents between the ages of 14–19 years old, we observed an inverse relationship between lifetime reported well arsenic concentration and endothelial function, as measured by RHI. Overall, individuals who reported drinking exclusively from wells with levels of arsenic >50 μg/L had significantly reduced endothelial function, as indicated by lower RHI, compared to individuals who drank exclusively from wells with ≤50 μg/L arsenic. Similar, albeit non-statistically significant trends, were observed for indivudals with moderate levels of lifetime arsenic exposure as well. Sex-stratified analyses suggested that these associations were stronger in female participants, with statistically significant reductions in RHI for female, but not male, participants who reported drinking exclusively from wells with levels of arsenic >50 μg/L, when compared to those who drank exclusively from wells with ≤50 μg/L. Overall, lower RHI scores were associated with greater well arsenic exposure, particularly among those who reported lifetime consumption of water from red wells, indicating levels of arsenic >50 μg/L.

Experimental studies have shown that arsenic exposure can alter endothelial function[44–47], but few epidemiologic studies have directly evaluated this association. Several studies have observed largely consistent associations between arsenic exposure and biomarkers of inflammation and endothelial function. For example, evidence from a small study among adults living in an As-endemic area of inner Mongolia suggested that chronic arsenic exposure was related to reduced nitric oxide production by endothelial cells, potentially indicative of lower vascular responsiveness.[48] Prior work from Araihazar, Bangladesh among adults found associations of water arsenic and urinary arsenic with greater levels of soluble circulating markers of endothelial function and inflammation, including intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), at baseline, as well as increased levels over time.[23, 24] Similar trends have been observed among individuals living in New Hampshire (NH) USA, who are primarily to exposed to low to moderate levels of arsenic via private unregulated wells. One study of NH adults observed that toenail arsenic levels were associated higher VCAM-1, and urinary arsenic levels were related to urinary oxidative stress marker 15-F_{2t}-isoprostane. Another study from NH that focused on pregnant women and their infants found that maternal urinary arsenic during

pregnancy was associated with higher levels of maternal plasma VCAM-1 in pregnancy, as well as higher levels of ICAM-1 and VCAM-1 in newborn cord blood.[34] However, to our knowledge, only one other study has examined arsenic exposure in relation to a functional measure of endothelial function. In recent work from the Multi-Ethnic Study of Atherosclerosis, investigators examined rice intake as a proxy for arsenic exposure in over 5000 adults.[35] Although researchers observed positive associations of rice intake with biomarkers of inflammation and endothelial function, including E-selectin and ICAM-1, they did not observe any association with endothelial function, as measured by brachial flow-mediation dilation, nor other subclinical measures of CVD. It is possible the observed lack of association with subclinical markers of CVD may be due in part to the method of arsenic exposure characterization. While rice intake may be a primary source of arsenic exposure in the MESA population, content of arsenic in rice varies greatly by the sources of the rice which is not captured in food-frequency questionnaires, potentially leading to substantial measurement error.[35, 49] Overall, several studies have identified associations between arsenic exposure and biomarkers of potential endothelial dysfunction and further work is needed to understand the relationship of arsenic to functional measures of vascular responsiveness.

We also observed sex-specific differences in RHI suggesting that chronic exposure to higher levels of well water arsenic may be related to greater reductions in RHI in females, as compared to males. Among these adolescents, statistically significant reductions in RHI were observed for female, but not male, participants who reported drinking exclusively from wells with levels of arsenic >50 μg/L, as compared to those who drank exclusively from wells with 50 μg/L arsenic. While limited, prior epidemiological studies of cardiovascular outcomes also have observed stronger effects among women.[50, 51] In Spain, cardiovascular disease and coronary heart disease mortality rates were somewhat higher among women exposed to low-to-moderate levels of arsenic via drinking water.[50] Among arsenic-exposed adults in Inner Mongolia, chronic arsenic exposure was associated with QT interval prolongation, with greater effects observed among female participants.[51] While the role of sex hormones in cardiovascular health is complex and may be influenced by a number of factors, females are often considered to be less susceptible to adverse cardiovascular effects, due to the generally cardioprotective nature of estrogen prior to menopause and prior studies have suggested that estrogen may modulate vasomotor tone and endothelial function.[52, 53] However, it is possible that arsenic exposure may disrupt estrogen-receptor signaling, as suggested by some experimental studies.[54, 55] One study found that among mice exposed to inorganic arsenic, female mice exhibited significant ischemia-reperfusion injury in the heart, while male mice did not, suggesting that females may be more susceptible to the endocrine disrupting effects of arsenic, particularly in relation to cardiovascular health effects.[56] However, additional research is needed to elucidate the role of arsenic exposure in endocrine disruption and the implications for sex-related differences in cardiovascular health outcomes.

While clinical manifestations of CVD do not often appear until adulthood, early indicators of these pathogenic processes can be measured in childhood.[57] For instance, atherosclerosis is a process with a long asymptomatic period that is detectable in children, though often overlooked.[58] Given the strong associations observed for arsenic exposure and CVD in adult

populations, a growing number of studies have begun to look toward younger populations to understand how arsenic may influence cardiovascular health in early life. To date, a handful of studies have observed associations between arsenic exposure and cardiovascular-related outcomes in children and adolescents.[19–22] Among 1,887 children in Bangladesh, *in utero* exposure to arsenic was associated with elevated BP at 4.5 years of age.[19] In two cross-sectional studies of children in Mexico, urinary arsenic was associated with increases in several cardiovascular risk factors, including carotid intima-media thickness (cIMT), asymmetric dimethylarginine levels, blood pressure, left ventricular mass and ejection fraction.[20, 21] A recent analysis of a group of 726 14–17 year old adolescents whose mothers were participants in the HEALS (separate from the individuals assessed in the current study), found that current arsenic exposure and early childhood arsenic exposure were associated with higher blood pressure and effects may be heightened among those with higher BMI.[22] We did not find an association of arsenic with blood pressure in the current study and blood pressure was only marginally correlated with RHI. Further, we did not observe any effect modification by BMI, therefore these differences in findings may be due in part to the method of exposure assessment and that well color recall is a less precise measure of arsenic exposure than urinary biomarkers of arsenic exposure used in prior studies. While these studies explored various cardiovascular-related outcomes, the overall evidence presented by prior studies and the current study suggests arsenic exposure in early life and childhood may be related to risk factors in childhood and adolescence that have been associated with adverse cardiovascular health outcomes in later life.

To our knowledge, ours is among the first to investigate a functional measure of vascular responsiveness in relation to arsenic exposure in adolescents. Our study has several strengths. The characterization of a functional measure of endothelial dysfunction during adolescence, a critical lifestage for long-term cardiovascular health, is a primary strength of this study. We utilized the non-invasive Endo-PAT 2000, which provides an objective measurement of RHI with a user-independent device that can be used with minimal training and is advantageous to conventional ultrasound-based techniques for assessing endothelial function, such as flow-mediated dilation, which traditionally are highly operator-dependent.[59] Prior studies have successfully used the Endo-PAT 2000 to examine endothelial function in adolescent and pediatric populations[60–66], including a large prospective study of children's health.[67] Further, EndoPAT-measured RHI has been previously used in the context of environmental exposures and RHI has been related to other cardiovascular-related pollutants, including air pollutants and metals.[68–72] Another strength of this study is that given the local infrastructure and well color coding in our study area, we were able to obtain information to estimate individual lifetime well water arsenic exposure, allowing for a comprehensive assessment of past and current exposures. Our study also has some limitations. At the time of this study, we lacked information on biomarkers of arsenic exposure, such as urinary or toenail arsenic. Individual characterization of exposure biomarkers could provide a fuller picture of both exposure, as well as the potential role of arsenic metabolism on endothelial function. Further, we relied on participants and their mothers to recall past drinking well colors, as well as length of time drinking from each well, in order to estimate lifetime arsenic exposure. It is possible that errors in recall may have influenced our observed associations. However, we would predict more accurate

recall among those with little to no variation in well color over time (approximately 69% of our sample reported using the same well over their lifetime), potentially leading to more accurate recall among those who drank only from a single well over their lifetime. This may have differentially influenced our ability to characterize exposures among those who drank from numerous wells over their lifetime, although the highest number of wells recorded over any participant's lifetime was three. Lastly, we cannot rule out the possibility of confounding due to unmeasured variables, such as dietary and lifestyle factors, such as level of physical activity, and co-exposures to other environmental pollutants, including air pollution and environmental tobacco smoke. For example, although we investigated the role of smoking in the home, we did not collect information about frequency of exposures or of the potential for environmental tobacco smoke outside the home, which could have influenced our results.

CVD accounts for one-third of total worldwide mortality, or ~17 million deaths yearly, and is expected to rise to >23 million by 2030.[73] Therefore, even small increases in CVD morbidity and mortality due to arsenic exposure would have a widespread public health impact. Our current understanding of the effects of arsenic exposure on CVD is largely limited to studies of adult populations and studies of early life effects among children and adolescents are lacking. Given that RHI may indicate an earlier stage of cardiovascular pathogenesis in adolescents, work including measures of endothelial function could help to identify apparently healthy adolescents who may be at higher risk of later life CVD, providing important intervention opportunities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Grant support:

References

1. WHO, W.H.O., Guidelines for drinking-water quality, fourth edition 2011.

2. International Agency for Research on Cancer, I., IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Drinking-water Disinfectants and Contaminants, including Arsenic. 2004: Lyon, France.

3. National Research Council, N., Critical Aspects of EPA's IRIS Assessment of Inorganic Arsenic: Interim Report. 2014, National Research Council: Washington, DC.

4. Agency for Toxic Substances and Disease, A., Toxicological Profile for Arsenic. 2007, US Dept of Health and Human Services.

5. Naujokas MF, Anderson B, Ahsan H, Aposhian HV, Graziano JH, Thompson C, et al. , The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. Environ Health Perspect, 2013. 121(3): p. 295–302. [PubMed: 23458756]

6. WHO, W.H.O., A global brief on hypertension: Silent killer, global public health crisis. 2013: Geneva, Switzerland.

7. EPA, IRIS Toxicological Review of Inorganic Arsenic (Cancer) (2010 External Review Draft). Washington, DC:U.S. Environmental Protection Agency (updated 20 June 2013). Available: http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=219111 [accessed 24 April 2014].

8. Chen CJ, Hsueh YM, Lai MS, Shyu MP, Chen SY, Wu MM, et al. , Increased prevalence of hypertension and long-term arsenic exposure. Hypertension, 1995. 25(1): p. 53–60. [PubMed: 7843753]

9. Kwok RK, Mendola P, Liu ZY, Savitz DA, Heiss G, Ling HL, et al. , Drinking water arsenic exposure and blood pressure in healthy women of reproductive age in Inner Mongolia, China. Toxicology and applied pharmacology, 2007. 222(3): p. 337–43. [PubMed: 17509635]

10. Chen Y, Graziano JH, Parvez F, Liu M, Slavkovich V, Kalra T, et al. , Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. BMJ, 2011. 342: p. d2431. [PubMed: 21546419]

11. Wu MM, Kuo TL, Hwang YH, and Chen CJ, Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. Am J Epidemiol, 1989. 130(6): p. 1123–32. [PubMed: 2589305]

12. Chen Y, Factor-Litvak P, Howe GR, Graziano JH, Brandt-Rauf P, Parvez F, et al. , Arsenic exposure from drinking water, dietary intakes of B vitamins and folate, and risk of high blood pressure in Bangladesh: a population-based, cross-sectional study. Am J Epidemiol, 2007. 165(5): p. 541–52. [PubMed: 17164464]

13. Wang S-L, Chang F-H, Liou S-H, Wang H-J, Li W-F, and Hsieh DPH, Inorganic arsenic exposure and its relation to metabolic syndrome in an industrial area of Taiwan. Environment International, 2007. 33(6): p. 805–811. [PubMed: 17481731]

14. Moon K, Guallar E, and Navas-Acien A, Arsenic exposure and cardiovascular disease: an updated systematic review. Curr Atheroscler Rep, 2012. 14(6): p. 542–55. [PubMed: 22968315]

15. Abhyankar LN, Jones MR, Guallar E, and Navas-Acien A, Arsenic exposure and hypertension: a systematic review. Environmental Health Perspectives, 2012. 120(4): p. 494–500. [PubMed: 22138666]

16. Tseng CH, Chong CK, Chen CJ, and Tai TY, Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. Atherosclerosis, 1996. 120(1–2): p. 125–33. [PubMed: 8645353]

17. Tseng CH, Chong CK, Tseng CP, and Centeno JA, Blackfoot disease in Taiwan: its link with inorganic arsenic exposure from drinking water. Ambio, 2007. 36(1): p. 82–4. [PubMed: 17408196]

18. Wu F, Molinaro P, and Chen Y, Arsenic Exposure and Subclinical Endpoints of Cardiovascular Diseases. Curr Environ Health Rep, 2014. 1(2): p. 148–162. [PubMed: 25013752]

19. Hawkesworth S, Wagatsuma Y, Kippler M, Fulford AJ, Arifeen SE, Persson LA, et al. , Early exposure to toxic metals has a limited effect on blood pressure or kidney function in later childhood, rural Bangladesh. Int J Epidemiol, 2012.

20. Osorio-Yanez C, Ayllon-Vergara JC, Aguilar-Madrid G, Arreola-Mendoza L, Hernandez-Castellanos E, Barrera-Hernandez A, et al. , Carotid Intima-Media Thickness and Plasma Asymmetric Dimethylarginine in Mexican Children Exposed to Inorganic Arsenic. Environ Health Perspect, 2013.

21. Osorio-Yanez C, Ayllon-Vergara JC, Arreola-Mendoza L, Aguilar-Madrid G, Hernandez-Castellanos E, Sanchez-Pena LC, et al. , Blood pressure, left ventricular geometry, and systolic function in children exposed to inorganic arsenic. Environ Health Perspect, 2015. 123(6): p. 629–35. [PubMed: 25738397]

22. Chen Y, Wu F, Liu X, Parvez F, LoIacono NJ, Gibson EA, et al. , Early life and adolescent arsenic exposure from drinking water and blood pressure in adolescence. Environ Res, 2019. 178: p. 108681. [PubMed: 31520830]

23. Wu F, Jasmine F, Kibriya MG, Liu M, Wojcik O, Parvez F, et al. , Association between arsenic exposure from drinking water and plasma levels of cardiovascular markers. Am J Epidemiol, 2012. 175(12): p. 1252–61. [PubMed: 22534204]

24. Chen Y, Santella RM, Kibriya MG, Wang Q, Kappil M, Verret WJ, et al. , Association between arsenic exposure from drinking water and plasma levels of soluble cell adhesion molecules. Environ Health Perspect, 2007. 115(10): p. 1415–20. [PubMed: 17938729]

25. Burgess JL, Kurzius-Spencer M, O'Rourke MK, Littau SR, Roberge J, Meza-Montenegro MM, et al. , Environmental arsenic exposure and serum matrix metalloproteinase-9. J Expo Sci Environ Epidemiol, 2013. 23(2): p. 163–9. [PubMed: 23232971]

26. Soucy NV, Mayka D, Klei LR, Nemec AA, Bauer JA, and Barchowsky A, Neovascularization and angiogenic gene expression following chronic arsenic exposure in mice. Cardiovasc Toxicol, 2005. 5(1): p. 29–41. [PubMed: 15738583]

27. Hays AM, Lantz RC, Rodgers LS, Sollome JJ, Vaillancourt RR, Andrew AS, et al. , Arsenic-induced decreases in the vascular matrix. Toxicol Pathol, 2008. 36(6): p. 805–17. [PubMed: 18812580]

28. Cai Z, Zhang Y, Zhang Y, Miao X, Li S, Yang H, et al. , Use of a Mouse Model and Human Umbilical Vein Endothelial Cells to Investigate the Effect of Arsenic Exposure on Vascular Endothelial Function and the Associated Role of Calpains. Environ Health Perspect, 2019. 127(7): p. 77003. [PubMed: 31274337]

29. Yu CX, Zhang YY, Wu XY, Tang HX, Liang XQ, Xue ZM, et al. , Transient receptor potential melastatin 4 contributes to early-stage endothelial injury induced by arsenic trioxide. Toxicol Lett, 2019. 312: p. 98–108. [PubMed: 31054354]

30. Straub AC, Stolz DB, Vin H, Ross MA, Soucy NV, Klei LR, et al. , Low level arsenic promotes progressive inflammatory angiogenesis and liver blood vessel remodeling in mice. Toxicol Appl Pharmacol, 2007. 222(3): p. 327–36. [PubMed: 17123562]

31. Vanhoutte PM, Endothelial dysfunction: the first step toward coronary arteriosclerosis. Circ J, 2009. 73(4): p. 595–601. [PubMed: 19225203]

32. Deanfield JE, Halcox JP, and Rabelink TJ, Endothelial function and dysfunction: testing and clinical relevance. Circulation, 2007. 115(10): p. 1285–95. [PubMed: 17353456]

33. Hasibuzzaman MM, Hossain S, Islam MS, Rahman A, Anjum A, Hossain F, et al. , Association between arsenic exposure and soluble thrombomodulin: A cross sectional study in Bangladesh. PLoS One, 2017. 12(4): p. e0175154. [PubMed: 28399171]

34. Farzan SF, Brickley EB, Li Z, Gilbert-Diamond D, Gossai A, Chen Y, et al. , Maternal and infant inflammatory markers in relation to prenatal arsenic exposure in a U.S. pregnancy cohort. Environ Res, 2017. 156: p. 426–433. [PubMed: 28410520]

35. Sobel MH, Sanchez TR, Jones MR, Kaufman JD, Francesconi KA, Blaha MJ, et al. , Rice Intake, Arsenic Exposure, and Subclinical Cardiovascular Disease Among US Adults in MESA. J Am Heart Assoc, 2020. 9(4): p. e015658. [PubMed: 32067593]

36. Tomfohr LM, Martin TM, and Miller GE, Symptoms of depression and impaired endothelial function in healthy adolescent women. J Behav Med, 2008. 31(2): p. 137–43. [PubMed: 18165894]

37. Liu J, Wang J, Jin Y, Roethig HJ, and Unverdorben M, Variability of peripheral arterial tonometry in the measurement of endothelial function in healthy men. Clin Cardiol, 2009. 32(12): p. 700–4. [PubMed: 20027662]

38. Ahsan H, Chen Y, Parvez F, Argos M, Hussain AI, Momotaj H, et al. , Health Effects of Arsenic Longitudinal Study (HEALS): description of a multidisciplinary epidemiologic investigation. J Expo Sci Environ Epidemiol, 2006. 16(2): p. 191–205. [PubMed: 16160703]

39. van Geen A, Ahmed EB, Pitcher L, Mey JL, Ahsan H, Graziano JH, et al. , Comparison of two blanket surveys of arsenic in tubewells conducted 12 years apart in a 25 km(2) area of Bangladesh. Sci Total Environ, 2014. 488–489: p. 484–92.

40. Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr., Kuvin JT, and Lerman A, Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. J Am Coll Cardiol, 2004. 44(11): p. 2137–41. [PubMed: 15582310]

41. Dhindsa M, Sommerlad SM, DeVan AE, Barnes JN, Sugawara J, Ley O, et al. , Interrelationships among noninvasive measures of postischemic macro- and microvascular reactivity. Journal of Applied Physiology, 2008. 105(2): p. 427–432. [PubMed: 18483158]

42. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. , Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. Am Heart J, 2003. 146(1): p. 168–74. [PubMed: 12851627]

43. Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, et al. , Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation, 2008. 117(19): p. 2467–74. [PubMed: 18458169]

44. Hou YC, Hsu CS, Yeh CL, Chiu WC, Pai MH, and Yeh SL, Effects of glutamine on adhesion molecule expression and leukocyte transmigration in endothelial cells exposed to arsenic. J Nutr Biochem, 2005. 16(11): p. 700–4. [PubMed: 16084078]

45. Lee MY, Jung BI, Chung SM, Bae ON, Lee JY, Park JD, et al. , Arsenic-induced dysfunction in relaxation of blood vessels. Environ Health Perspect, 2003. 111(4): p. 513–7. [PubMed: 12676608]

46. Jindal S, Singh M, and Balakumar P, Effect of bis (maltolato) oxovanadium (BMOV) in uric acid and sodium arsenite-induced vascular endothelial dysfunction in rats. Int J Cardiol, 2008. 128(3): p. 383–391. [PubMed: 17658639]

47. Ellinsworth DC, Arsenic, reactive oxygen, and endothelial dysfunction. J Pharmacol Exp Ther, 2015. 353(3): p. 458–64. [PubMed: 25788710]

48. Pi J, Kumagai Y, Sun G, Yamauchi H, Yoshida T, Iso H, et al. , Decreased serum concentrations of nitric oxide metabolites among Chinese in an endemic area of chronic arsenic poisoning in inner Mongolia. Free Radic Biol Med, 2000. 28(7): p. 1137–42. [PubMed: 10832076]

49. Jackson BP, Taylor VF, Karagas MR, Punshon T, and Cottingham KL, Arsenic, organic foods, and brown rice syrup. Environ Health Perspect, 2012. 120(5): p. 623–6. [PubMed: 22336149]

50. Medrano MA, Boix R, Pastor-Barriuso R, Palau M, Damian J, Ramis R, et al. , Arsenic in public water supplies and cardiovascular mortality in Spain. Environ Res, 2010. 110(5): p. 448–54. [PubMed: 19880104]

51. Mumford JL, Wu K, Xia Y, Kwok R, Yang Z, Foster J, et al. , Chronic arsenic exposure and cardiac repolarization abnormalities with QT interval prolongation in a population-based study. Environ Health Perspect, 2007. 115(5): p. 690–4. [PubMed: 17520054]

52. Stanhewicz AE, Wenner MM, and Stachenfeld NS, Sex differences in endothelial function important to vascular health and overall cardiovascular disease risk across the lifespan. Am J Physiol Heart Circ Physiol, 2018. 315(6): p. H1569–H1588. [PubMed: 30216121]

53. Riedel K, Deussen AJ, Tolkmitt J, Weber S, Schlinkert P, Zatschler B, et al. , Estrogen determines sex differences in adrenergic vessel tone by regulation of endothelial beta-adrenoceptor expression. Am J Physiol Heart Circ Physiol, 2019. 317(2): p. H243–H254. [PubMed: 31149843]

54. Munoz A, Chervona Y, Hall M, Kluz T, Gamble MV, and Costa M, Sex-specific patterns and deregulation of endocrine pathways in the gene expression profiles of Bangladeshi adults exposed to arsenic contaminated drinking water. Toxicol Appl Pharmacol, 2015. 284(3): p. 330–8. [PubMed: 25759245]

55. Davey JC, Bodwell JE, Gosse JA, and Hamilton JW, Arsenic as an endocrine disruptor: effects of arsenic on estrogen receptor-mediated gene expression in vivo and in cell culture. Toxicol Sci, 2007. 98(1): p. 75–86. [PubMed: 17283378]

56. Veenema R, Casin KM, Sinha P, Kabir R, Mackowski N, Taube N, et al. , Inorganic arsenic exposure induces sex-disparate effects and exacerbates ischemia-reperfusion injury in the female heart. Am J Physiol Heart Circ Physiol, 2019. 316(5): p. H1053–H1064. [PubMed: 30822117]

57. Center, L.S.U.M., M. Clinic, U.o.I. Hospitals, Clinics, L. National Heart, and B. Institute, Cardiovascular Profile of 15,000 Children of School Age in Three Communities, 1971–1975. 1978: U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute.

58. Berenson GS, Srinivasan SR, Hunter SM, Nicklas TA, Freedman DS, Shear CL, et al. , Risk factors in early life as predictors of adult heart disease: the Bogalusa Heart Study. Am J Med Sci, 1989. 298(3): p. 141–51. [PubMed: 2679086]

59. Bonetti PO, Barsness GW, Keelan PC, Schnell TI, Pumper GM, Kuvin JT, et al. , Enhanced external counterpulsation improves endothelial function in patients with symptomatic coronary artery disease. J Am Coll Cardiol, 2003. 41(10): p. 1761–8. [PubMed: 12767662]

60. Kelly AS, Metzig AM, Rudser KD, Fitch AK, Fox CK, Nathan BM, et al. , Exenatide as a weight-loss therapy in extreme pediatric obesity: a randomized, controlled pilot study. Obesity (Silver Spring), 2012. 20(2): p. 364–70. [PubMed: 22076596]

61. Kelly AS, Metzig AM, Steinberger J, and Braunlin EA, Endothelial function in children and adolescents with mucopolysaccharidosis. J Inherit Metab Dis, 2013. 36(2): p. 221–5. [PubMed: 22231383]

62. Metzig AM, Schwarzenberg SJ, Fox CK, Deering MM, Nathan BM, and Kelly AS, Postprandial Endothelial Function, Inflammation, and Oxidative Stress in Obese Children and Adolescents. Obesity, 2011. 19(6): p. 1279–1283. [PubMed: 21233813]

63. Tryggestad JB, Thompson DM, Copeland KC, and Short KR, Obese Children Have Higher Arterial Elasticity Without a Difference in Endothelial Function: The Role of Body Composition. Obesity, 2012. 20(1): p. 165–171. [PubMed: 21996664]

64. Kheirandish-Gozal L, Etzioni T, Bhattacharjee R, Tan HL, Samiei A, Molero Ramirez H, et al. , Obstructive sleep apnea in children is associated with severity-dependent deterioration in overnight endothelial function. Sleep Med, 2013. 14(6): p. 526–31. [PubMed: 23643649]

65. Chen Y, Osika W, Dangardt F, Gan LM, Strandvik B, and Friberg P, High levels of soluble intercellular adhesion molecule-1, insulin resistance and saturated fatty acids are associated with endothelial dysfunction in healthy adolescents. Atherosclerosis, 2010. 211(2): p. 638–42. [PubMed: 20362293]

66. Radtke T, Kriemler S, Eser P, Saner H, and Wilhelm M, Physical activity intensity and surrogate markers for cardiovascular health in adolescents. Eur J Appl Physiol, 2013. 113(5): p. 1213–22. [PubMed: 23160655]

67. Quante M, Hesse M, Dohnert M, Fuchs M, Hirsch C, Sergeyev E, et al. , The LIFE child study: a life course approach to disease and health. BMC Public Health, 2012. 12: p. 1021. [PubMed: 23181778]

68. Bugge MD, Ulvestad B, Berlinger B, Stockfelt L, Olsen R, and Ellingsen DG, Reactive hyperemia and baseline pulse amplitude among smelter workers exposed to fine and ultrafine particles. International Archives of Occupational and Environmental Health, 2020. 93(3): p. 399–407. [PubMed: 31773255]

69. Erqou S, Clougherty J, and Reis S, The Role Of Exposure To Environmental Pollutants In Racial Disparity In Cardiovascular Risk Factors And Novel Markers. Circulation, 2016. 134.

70. McGraw KE, Riggs DW, Rai S, Navas-Acien A, Xie ZZ, Lorkiewicz P, et al. , Exposure to volatile organic compounds-acrolein, 1,3-butadiene, and crotonaldehyde-is associated with vascular dysfunction. Environmental Research, 2021. 196.

71. Mentz RJ and O'Brien EC, Air Pollution in Patients With Heart Failure: Lessons From a Mechanistic Pilot Study of a Filter Intervention. JACC Heart Fail, 2016. 4(1): p. 65–7. [PubMed: 26738953]

72. Shahriar MH, Chowdhury MAH, Ahmed S, Eunus M, Kader SB, Begum BA, et al. , Exposure to household air pollutants and endothelial dysfunction in rural Bangladesh: A cross-sectional study. Environ Epidemiol, 2021. 5(2): p. e132. [PubMed: 33870008]

73. WHO, W.H.O., Causes of Death 2008. 2008: Geneva, Switzerland.

Table 1.

Selected characteristics of 200 adolescent participants with endothelial function measurement, overall and categorized by lifetime well As level.

Participant Characteristics	Overall	Always blue (<10 μg/L) or blue and green (10–50 μg/L) (N=28)	Always green (10–50 μg/L) or ever red (>50 μg/L) (N=93)	Always red (>50 μg/L) (N=79)	p-value
		Mean (SD) or N (%)			
Sex					
Male	106 (53.0)	10 (35.7)	51 (54.8)	45 (57.0)	0.14
Female	94 (47.0)	18 (64.3)	42 (45.2)	34 (43.0)	
Age, years	17.4 (0.9)	17.6 (1.0)	17.4 (0.9)	17.4 (0.9)	0.56
Height, cm	159.0 (9.5)	156.8 (8.3)	159.2 (9.5)	159.4 (9.8)	0.84
Weight, kg	50.3 (9.0)	50.8 (10.2)	50.1 (8.6)	50.2 (9.2)	0.94
BMI, kg/m^2	20.6 (3.6)	21.6 (4.6)	20.5 (3.5)	20.4 (3.3)	0.29
Attending School					
Yes	141 (70.5)	23 (82.1)	68 (73.1)	50 (63.3)	0.13
No	59 (29.5)	5 (17.9)	25 (26.9)	29 (36.7)	
Education level					
No education	7 (3.5)	0 (0.0)	4 (4.3)	3 (3.8)	0.16
Primary (up to 5th grade)	25 (12.5)	4 (14.3)	6 (6.5)	15 (19.0)	
Secondary (6–10th grade)	91 (45.5)	10 (35.7)	46 (49.5)	35 (44.3)	
Higher secondary (11–12th grade)	74 (37.0)	13 (46.4)	35 (37.6)	26 (32.9)	
Graduate (>12th grade)	3 (1.5)	1 (3.6)	2 (2.1)	0 (0.0)	
Current Smoker					
Yes	4 (2.0)	1 (3.6)	3 (3.2)	0 (0.0)	0.25
No	196 (98.0)	27 (96.4)	90 (96.8)	79 (100.0)	
Smoker in household					
Yes	101 (50.5)	10 (35.7)	54 (58.1)	37 (46.8)	0.08
No	99 (49.5)	18 (64.3)	39 (41.9)	42 (53.2)	
Maternal Education, years[a]	4.2 (3.9)	5.6 (3.6)	4.2 (4.1)	3.7 (3.8)	0.09
Maternal Land Ownership[a]					

	Overall	Always blue (<10 µg/L) or blue and green (10–50 µg/L) (N=28)	Always green (10–50 µg/L) or ever red (>50 µg/L) (N=93)	Always red (>50 µg/L) (N=79)	p-value
Yes	119 (59.8)	21 (75.0)	53 (57.0)	45 (57.7)	0.21
No	80 (40.2)	7 (25.0)	40 (43.0)	33 (42.3)	
Systolic Blood Pressure, mmHg	111.1 (11.5); range 86.5–146.5	114.8 (11.6); range: 91.5–146.5	111.4 (11.4); range: 86.5–142.5	109.4 (11.4) range: 87.0–138.5	0.10
Diastolic Blood Pressure, mmHg	68.2 (7.0); range 50.0–86.0	70.1 (6.4) range: 59.0–84.0	68.1 (7.3); range: 50.0–86.0	67.5 (6.8); range: 53.0–82.5	0.24
Exposure Variables					
Current Well As Concentration					
<10 µg/L	30 (15.0)	17	13	0	--
10–50 µg/L	61 (30.5)	5	56	0	
>50 µg/L	73 (36.5)	0	10	63	
Unknown	36 (18.0)	6	14	16	
EndoPAT variables					
RHI	1.52 (0.43); range 0.78–3.28	1.63 (0.47); range: 1.05–3.22	1.51 (0.39); range: 0.78–2.68	1.48 (0.46) range: 0.79–3.28	0.28
lnRHI	0.38 (0.25); range: −0.25–1.19	0.45 (0.26); range: 0.05–1.17	0.38 (0.24); range: −0.25–0.99	0.35 (0.27); range: −0.24–1.19	0.21

[a]N=199 for maternal land ownership and maternal educational length variables due to missing information for one participant.

Table 2.

Associations between lifetime well As level and percent difference in endothelial function as measured by reactive hyperemia index (RHI), adjusted for covariates (N=199).

	N	% difference in RHI (95% CI)[a]	p-value	p-trend
Always blue (<10 μg/L) or blue and green (10–50 μg/L)	28	Ref.	--	
Always green (10–50 μg/L) or ever red (>50 μg/L)	93	−7.69 (−17.30, 3.25)	0.16	**0.03**
Always red (>50 μg/L)	78	−11.75 (−21.26, −1.09)	**0.03**	

[a]Model adjusted for participant sex, age, education level and weight at time of assessment and maternal education and land ownership. Percent difference in RHI estimated by back-transformation of beta estimate of natural log transformed reactive hyperemia index (lnRHI). Missing maternal education and land ownership for one participant reduced sample size to N=199.

Table 3.

Association between current well As[a] level and percent difference in endothelial function as measured by reactive hyperemia index (RHI), adjusted for covariates[b] (N=199).

	N	% difference in RHI (95% CI)[b]	p-value	p-trend
Blue (<10 µg/L)	38	Ref.	--	
Green (10–50 µg/L)	70	−3.82 (−13.30, 6.66)	0.45	0.13
Red (>50 µg/L)	91	−7.32 (−16.18, 2.43)	0.14	

[a]Current well level was imputed as most recent recalled well color if current well color was unknown at time of assessment.

[b]Model adjusted for participant sex, age, education level and weight at time of assessment and maternal education and land ownership. Percent difference in RHI estimated by back-transformation of beta estimate of natural log transformed reactive hyperemia index (lnRHI). Missing maternal education and land ownership for one participant reduced sample size to N=199.

Table 4.

Associations between lifetime well As level and percent difference in endothelial function as measured by reactive hyperemia index, stratified by sex.

Lifetime well color history	Males (N=105)				Females (N=94)			
	N	% difference in RHI (95% CI)	p-value	p-trend	N	% difference in RHI (95% CI)	p-value	p-trend
Always blue (<10 μg/L) or blue and green (10–50 μg/L)	10	Ref.	--		18	Ref.	--	
Always green (10–50 μg/L) or ever red (>50 μg/L)	51	−1.00 (−18.37, 20.08)	0.92	0.44	42	−10.33 (−21.96, 3.01)	0.12	**0.04**
Always red (>50 μg/L)	44	−5.35 (−22.28, 15.37)	0.58		34	**-14.36 (−25.69, −1.29)**	**0.03**	

Model adjusted for participant age, education level and weight at time of assessment and maternal education and land ownership. Percent difference in RHI estimated by back-transformation of beta estimate of natural log transformed reactive hyperemia index (lnRHI)