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Blood pressure hyperreactivity: an early cardiovascular risk in normotensive men exposed to low-to-moderate inorganic arsenic in drinking water

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Abstract

Essential hypertension is associated with chronic exposure to high levels of inorganic arsenic in drinking water. However, early signs of risk for developing hypertension remain unclear in people exposed to chronic low-to-moderate inorganic arsenic.

Objective—We evaluated cardiovascular stress reactivity and recovery in healthy, normotensive, middle-aged men living in an arsenic-endemic region of Romania.

Methods: Unexposed ($n=16$) and exposed ($n=19$) participants were sampled from communities based on WHO limits for inorganic arsenic in drinking water ($<10 \mu\text{g/l}$). Water sources and urine samples were collected and analyzed for inorganic arsenic and its metabolites. Functional evaluation of blood pressure included clinical, anticipatory, cold pressor test, and recovery measurements.

Results: Blood pressure hyperreactivity was defined as a combined stress-induced change in SBP ($>20 \text{ mmHg}$) and DBP ($>15 \text{ mmHg}$). Drinking water inorganic arsenic averaged 40.2 ± 30.4 and $1.0 \pm 0.2 \mu\text{g/l}$ for the exposed and unexposed groups, respectively ($P < 0.001$). Compared to the unexposed group, the exposed group expressed a greater probability of blood pressure hyperreactivity to both anticipatory stress (47.4 vs. 12.5%; $P = 0.035$) and cold stress (73.7 vs. 37.5%; $P = 0.044$). Moreover, the exposed group exhibited attenuated blood pressure recovery from stress and a greater probability of persistent hypertensive responses (47.4 vs. 12.5%; $P = 0.035$).

Conclusions: Inorganic arsenic exposure increased stress-induced blood pressure hyperreactivity and poor blood pressure recovery, including persistent hypertensive responses in otherwise healthy, clinically normotensive men. Drinking water containing even low-to-moderate inorganic arsenic may act as a sympathetic nervous system trigger for hypertension risk.

Keywords

hypertension; physiological stress reactivity; sympathetic nervous system

INTRODUCTION

The prevalence of hypertension (140/90 mmHg) and the associated risk for additional cardiovascular and renal disorders are important global health concerns [1]. An estimated 95% of all hypertension is considered essential hypertension with no single definitive cause [2]. An environmental factor that appears to contribute to the development of hypertension is inorganic arsenic (iAs).

The WHO lists arsenic on its 'top ten' list of chemicals for public health concern [3]. iAs is both a naturally occurring and an anthropogenically generated element found ubiquitously throughout the world. At high levels of exposure ($>300 \mu\text{g/l}$), iAs is known to have profound effects on the cardiovascular system [4,5], including increasing the prevalence of hypertension [6]. Globally, however, most drinking water sources contain low-to-moderate levels of iAs ($<300 \mu\text{g/l}$), presenting a common but uncertain health concern for physiological systems [4]. In addition, iAs was recently detected in foods containing organic brown rice syrup (a replacement for high-fructose corn syrup), demonstrating that our food supply is a vulnerable source of exposure [7]. The association between chronic low-to-moderate iAs exposure and risk for hypertension remains unclear: some studies report positive associations [8–10], whereas others conclude only mild effects [11], which may be due to regional sampling. Given its global occurrence, low-to-moderate iAs exposure is potentially an under-recognized environmental trigger for essential hypertension. Recognizing early warning signs for cardiovascular risk attributed to chronic consumption of low-to-moderate iAs may be important to lessening the burden of hypertension and cardiovascular disease.

An exaggerated blood pressure response to stress, or hyperreactivity, in normotensive individuals, is associated with increased risk for future hypertension and cardiovascular disease [12–20]. Evidence from animal models demonstrates that chronic consumption of iAs in drinking water causes increased sympathetically mediated blood pressure hyperreactivity [21], increased blood pressure, and left-ventricular hypertrophy (LVH) [22]. These physiological conditions are also observed in people with hypertension [23]. Moreover, a longitudinal sample of individuals with arsenosis, including those with skin lesions as well as Raynaud's syndrome, showed improved finger blood pressure reactivity to cold stress following remediation of high levels of arsenic in drinking water [24]. Accordingly, cold stress represents a simple physiological challenge that has the potential to unmask early signs of arsenic-induced cardiovascular risk, measured by sympathetic blood pressure reactivity [25]. Cold stress is also a practical assessment tool for rural, low-resource areas, where arsenic-contaminated drinking water may have a significant community impact.

The objective of this study was to investigate the effects of chronic low-to-moderate iAs exposure on susceptibility for hypertension in normotensive, middle-aged men living in rural Romania. Romania has stable, low-to-moderate iAs levels in drinking water ($<0.1\text{--}240 \mu\text{g/l}$), and these levels are among the highest observed aquifer levels of arsenic in Europe [26,27]. Rural communities still rely heavily on well water sources for drinking and cooking. Romania also has one of the highest cardiovascular risks in Europe, and men are at greater risk than women [28,29]. In addition, rural areas of Romania were recently identified to have a higher prevalence of hypertension than urban areas [29]. Causes for this difference are unknown, but rural as compared to urban inhabitants may have greater iAs exposure through drinking well water. Therefore, Romania is an important country in which to examine the impact of low-to-moderate iAs exposure on susceptibility for hypertension. To measure susceptibility, we used the cold pressor test (CPT) as a well accepted sympathetic nervous system challenge for blood pressure reactivity [25]. We hypothesized that chronic

low-to-moderate iAs exposure would increase the probability of blood pressure hyperreactivity to a CPT, indicating an early sign of risk for developing hypertension in otherwise healthy, normotensive men.

METHODS

Study population

A functional assessment of blood pressure reactivity was conducted in two rural towns in Arad County, Romania, one with low-to-moderate levels of arsenic exposure (exposed) and the other with negligible levels of arsenic exposure (unexposed). Individuals from the unexposed ($<2 \mu\text{g/l}$) and exposed ($>10 \mu\text{g/l}$) towns were chosen based on drinking water arsenic information collected in the Arsenic Health Risk Assessment and Molecular Epidemiology (ASHRAM) study [30]. Both towns had similar occupational and lifestyle profiles. Participants were recruited through the family doctor managing the care of the majority of inhabitants in each town. All participants used water sources representative of the communities. Participants were required to be 30–55 years of age and have a longstanding residence (>20 years) in the locality with no arsenic-related occupational hazards. Importantly, participants were required to have a stable, normotensive clinical blood pressure over the previous 2 years as well as no known personal history of hypertension, diabetes, cancer, or diseases of the cardiovascular system, liver, or kidney. Demographic information for the study population is presented in Table 1.

Assessments were conducted on-site in the rural clinics familiar to participants. The participants completed a study visit consisting of an interview conducted in Romanian by a trained interviewer, height and weight measurements, and functional stress testing to assess blood pressure reactivity and recovery responses. The interview assessed water consumption, occupational exposures, health history, and lifestyle habits. The clinical blood pressure value and family history of hypertension for each participant were obtained from medical records. All study participants gave written informed consent. The protocol was approved by the institutional review boards of the regional public health authority of Arad County in Romania, as well as the Human Investigation Committee for Yale University School of Medicine.

Water samples

Individual exposure assessment was conducted by collecting water samples from the current main drinking water source for each participant. Samples were collected and analyzed by standard methods [30]. Arsenic measurements that fell below the detection limit were imputed as the detection limit of $1 \mu\text{g/l}$.

Urine samples

A spot urine sample was collected to measure arsenic urinary metabolites at the time of stress testing. Spot urine for metabolites has been shown to have strong intra-individual reproducibility over several days of assessment [31]. Urine samples were collected and analyzed by standard methods for dimethylarsinic acid (DMA^{V}), monomethylarsonic acid (MMA^{V}), and total iAs [30]. Additionally, a portion of the urine was oxidized with H_2O_2 to convert any trivalent and thio-arsenicals to their pentavalent and/or oxygenated forms. Arsenic measurements were adjusted for dilution variation using average specific gravity in the sample population. Arsenic measurements that fell below the detection limit were imputed as the detection limit of $0.1 \mu\text{g/l}$. The primary methylation index (PMI) was calculated as the ratio of $\text{MMA}^{\text{V}}/\text{iAs}$ and the secondary methylation index (SMI) as the ratio of $\text{DMA}^{\text{V}}/\text{MMA}^{\text{V}}$.

Anticipatory stress and cold pressor test

The CPT was modeled after Hines's original protocol [32], which included the anticipatory stress phase characterized later by Gregg *et al.* [33]. Clinical blood pressure values obtained from medical records were used as the baseline for anticipatory (anticipation of the CPT) and cold stressors. Participants were instructed by the clinical staff to avoid smoking, alcohol, tea, coffee, and strenuous exercise 2 h before the study visit. After 20 min of seated rest during the interview and an additional 5 min in the supine position, anticipatory and recovery-from-anticipation blood pressures and heart rates were taken 5 min apart with participants in the supine position. They then underwent CPT, which involved immersing the left hand up to the wrist in ice water ($4^{\circ}\text{C}\pm 1^{\circ}\text{C}$) for 1 min. Recovery-from-cold blood pressure and heart rate were measured at the end of cold immersion and after 2, 5, and 10 min following hand removal from the ice water. All measurements were taken at the right brachial artery using an automated sphygmomanometer (Hartman Tensoval M2; clinically recognized as valid by British Hypertension Society). A blood pressure reaction to stress was considered hyperreactive if both the changes in SBP and DBP in response to the cold stress were above 20 and 15 mmHg, respectively [32]. The average of all recovery measurements was used as the recovery blood pressure value. A blood pressure response was considered hypertensive if it was at least 140/90 mmHg.

Statistical analysis

Data were analyzed using IBM SPSS version 19 (SPSS Inc., and IBM Company, IBM Corporation, USA). Study population characteristics, drinking water arsenic exposure, and urinary arsenic metabolites were compared between exposure groups and tested for significance ($\alpha=0.05$) using the student *t*-test, Fisher's exact test, and multivariate ANOVA analysis when appropriate. Data were tested for normality, and repeated-measures ANOVA analysis was performed with clinical, anticipatory and cold stressors, and recovery data in the model. Analysis included planned contrasts to the clinical blood pressure. Groups were compared for hyperreactive responses to stress, episodic hypertensive blood pressure ($> 140/90$ mmHg), and persistent hypertensive blood pressure ($> 140/90$ mmHg) using the Fisher's exact test. Binary logistic regression analysis was carried out for hyperreactivity and hypertensive blood pressure responses using exposure, BMI, family history of hypertension, and smoking as independent variables.

RESULTS

The exposed and unexposed groups had similar levels of primary risk characteristics, such as family history of hypertension and smoking (Table 1). Consistent with values expected from water mapping in the ASHRAM study [30], drinking water for exposed and unexposed participants had mean iAs levels of 40.2 ± 30.4 and $1.0\pm 0.2\mu\text{g/L}$, respectively ($P<0.001$) (Table 2). Whereas none of the unexposed participants had substantial iAs levels in their drinking water, all the exposed ones had water sources (range $15.3\text{--}132.4\mu\text{g/l}$) above the current acceptable limit of $10\mu\text{g/l}$ set by the European Environmental Agency, WHO, and US Environmental Protection Agency.

Urinary arsenic metabolites (Table 2) were highly correlated with water arsenic measurements, indicating parallels between external and internal measures of exposure (iAs: $r=0.697$, $P<0.001$; DMA^V: $r=0.812$, $P<0.001$; MMA^V: $r=0.606$, $P<0.001$). The exposed group had higher internal exposure compared to the unexposed one ($P<0.001$) (Table 2); however, unexposed and exposed participants exhibited similar levels of relative arsenic metabolic efficiency, assessed by examining the methylation indices PMI and SMI (Table 2).

Functional stress testing for blood pressure reactivity and recovery responses

There was a difference in response to anticipatory stress between the exposed and unexposed groups that mirrored the CPT response. Accordingly, the data were examined for both anticipatory (mental) and cold stress (physical) stress challenges to the cardiovascular system using the requisite stable, clinically normotensive blood pressure as the baseline. Baseline clinical SBP (unexposed: 132.3 ± 5.8 mmHg; exposed: 127.9 ± 8.9 mmHg; $P=0.101$) and DBP (unexposed: 76.9 ± 6.2 mmHg; exposed: 73.4 ± 6.2 mmHg; $P=0.112$) were not different between exposure groups.

Anticipatory stress

Anticipatory stress was defined as the stress experienced while participants anticipated the discomfort associated with the impending hand immersion into cold water. Mean blood pressure responses are presented in Fig. 1. There was a significant main effect for anticipatory stress ($P<0.015$) to increase both SBP (A) and DBP (B). There was also a significant interaction effect for DBP as a function of both the anticipatory stress and recovery from anticipatory stress ($P<0.050$). Importantly, while SBP returned to clinical baseline in the unexposed group, it remained elevated above clinical baseline in the exposed group ($P=0.006$). DBP remained elevated above baseline in both groups ($P<0.006$).

Cold stress

With respect to blood pressure responses, there were significant main effects for cold stress ($P<0.010$) and interactive effects ($P<0.035$) for both SBP (Fig. 2a) and DBP (Fig. 2b). As observed for anticipatory stress, recovery-from-cold SBP responses remained elevated from clinical baseline only in the exposed group ($P=0.004$). DBP remained elevated in both groups ($P<0.002$). Notably, after 10 minutes of recovery from cold immersion, 47.4% of the exposed group exhibited hypertensive blood pressures, whereas only 12.5% of the unexposed group was still at a hypertensive level ($P=0.035$).

Heart rate responses showed neither a significant main effect for group nor evidence for an interaction effect across stress challenges ($P>0.100$) (Table 3). There was a significant main effect for heart rate ($P=0.039$), but not as a planned contrast to clinical baseline.

Table 4 provides the average change in blood pressure for both stressors and recovery periods for each exposure group. Multivariate ANOVA analysis revealed significant between-group differences in SBP for cold stress ($P=0.033$) and recovery ($P=0.028$), and in DBP for all responses ($P<0.050$).

Blood pressure: hyperreactivity and hypertensive blood pressure responses

Stress is expected to increase blood pressure in healthy responders, though not in an exaggerated, hyperreactive manner. Moreover, any hypertensive level of blood pressure is expected to quickly resolve to normotensive levels after the stress has been removed. On the basis of the traditional clinical risk definitions, a larger percentage of exposed individuals exhibited hyperreactive blood pressure responses to both anticipatory stress (47.4 vs. 12.5%; $P=0.035$) and cold stress (73.7 vs. 37.5%; $P=0.044$), compared to unexposed individuals (Fig. 3a). Logistic regression revealed exposure to iAs to be a significant predictor for stress-induced blood pressure hyperreactivity [anticipatory stress: odds ratio (OR) 6.30, 95% confidence interval (CI) 1.11–35.67, $P=0.038$; cold stress: OR 4.67, 95% CI 1.11–19.65, $P=0.036$]. Family history of hypertension, BMI, and smoking were not significant predictors of blood pressure hyperreactivity ($P>0.050$).

For both anticipatory and cold stressors, we observed similar episodic hypertensive responses between groups (Fig. 3b; $P>0.100$). These were stress-induced hypertensive

responses that resolved during poststress recovery. Logistic regression revealed that BMI (OR 1.39, 95% CI 1.09–1.76, $P=0.007$) and smoking (OR 0.21, 95% CI 0.05–0.91, $P=0.037$) were the only significant predictors of an episodic hypertensive response to anticipatory and cold stress, respectively.

In contrast to the similarity of episodic hypertensive responses, persistent hypertensive responses (blood pressure responses remaining at a hypertensive level during stress and subsequent recovery) were more frequent in the exposed group compared to the unexposed group (Fig. 3c; anticipatory stress: 42.1 vs. 18.8%; $P=0.167$; cold stress: 47.4 vs. 12.5%; $P=0.035$). Notably, throughout all testing procedures, only one exposed participant (5.3%) remained normotensive, whereas 25% of the unexposed participants remained normotensive.

Consistent with the findings for blood pressure hyperreactivity, logistic regression revealed exposure status to be a significant predictor of a persistent hypertensive response to cold stress (OR 6.30, 95% CI 1.11–35.66, $P=0.038$). Family history of hypertension, BMI, and smoking were not significant predictors of a persistent hypertensive response to cold stress.

DISCUSSION

Our findings demonstrate, for the first time, that low-to-moderate iAs exposure in drinking water increases stress-induced blood pressure hyperreactivity and impairs blood pressure recovery from stress in otherwise healthy, normotensive, middle-aged men. These stress reactivity responses are considered early risk factors for the development of clinical hypertension. Moreover, the arsenic-exposed men had a higher probability of a hypertensive blood pressure response to cold stress persisting even after 10 min of recovery.

The early signs of risk for essential hypertension attributed to arsenic exposure at low-to-moderate levels of iAs exposure are unknown. Here we examined the impact of chronic low-to-moderate arsenic exposure on blood pressure reactivity in clinically normotensive men using a functional assessment of the cardiovascular response to stress and recovery elicited from a well accepted sympathetic nervous system challenge [25]. Previous research demonstrates the validity of gauging risk for developing hypertension through the assessment of blood pressure responses to stress. In multiple studies, higher risk for developing hypertension has been associated with the following physiological indicators: increased blood pressure reactivity to anticipatory and mental stress [12–15], increased blood pressure response to cold stress [16,17,20], and impaired blood pressure recovery following stress [12,18,19]. We observed dysfunctional responses under all these physiological conditions in the exposed group, providing evidence that iAs, or one of its metabolites, may disrupt the regulation of blood pressure. Therefore, our data present strong, novel evidence that iAs exposure at low-to-moderate concentrations may increase risk for developing hypertension, on the basis of stress reactivity [34] and stress recovery [12]. Our proof-of-concept study underscores the uniqueness and utility of a functional physiological stress paradigm to begin unmasking the effects of chronic low-to-moderate arsenic exposure on cardiovascular risk.

The magnitude of anticipatory stress in the exposed group was an unexpected outcome in our study. Like cold stress, anticipatory stress has been shown to increase peripheral vascular resistance [33]. Importantly, anticipatory stress reactivity was demonstrated to influence subsequent response to cold stimulation [33]. Therefore, we accounted for the full timeline of stress events, starting from anticipation of the stressor. Participants from both exposure groups had similar clinical blood pressure values; however, the efficient return to the baseline clinical blood pressure was only achieved by the unexposed group after both physiological stressors. These data lend validity to using the clinical blood pressure as the

baseline. Moreover, similarity between the study population's clinical blood pressures and recent Romanian population data [29] offer further confidence in this representative baseline measurement.

The significance of our anticipatory stress findings are demonstrated by a prospective study conducted in middle-aged, Finnish men ($n=580$). Men exhibiting greater reactivity to anticipatory stress had almost four times the risk for hypertension compared to men with lower reactivity [13]. In addition, a recent meta-analysis of prospective cardiovascular reactivity studies reported a 23% increased risk for future hypertension in individuals with higher blood pressure reactivity scores (generally considered as an elevation of at least 20 mmHg) during mental stress testing [12]. In our study, the exposed group had an average anticipatory stress elevation consistent with an increased risk for hypertension, as previously observed [12,13]. In contrast, the unexposed group demonstrated a lower response not indicative of increased risk. Furthermore, the probability of a hyperreactive response to anticipatory stress was significantly higher in the exposed group. These findings suggest that common daily stressors are more likely to impact individuals with low-to-moderate arsenic exposure.

The CPT represents a classic tool to examine blood pressure reactivity responses. The average normal reaction to cold stress is an elevation of 12 mmHg for SBP and 10 mmHg for DBP, whereas the upper range of normal is an elevation of 22 mmHg for SBP and 18 mmHg for DBP [35]. In our study, the reaction to cold stress for the unexposed group fell within the normal range, whereas that of the exposed group was 3.2 times (SBP) and 3.4 times (DBP) the normal response. Prospective studies of the CPT demonstrate that the risk of developing hypertension is increased for a mixed-sex sample of hyperreactors [16] and for an all-male sample of hyperreactors [17], as compared to normal reactors. In a 45-year follow-up study, 71% of hyperreactors and only 19% of normal reactors developed hypertension [20]. In our study, the exposed group had a significantly greater probability of exhibiting a hyperreactive blood pressure response to cold stress than the unexposed group and would therefore be considered at elevated risk for hypertension.

The exposed group of our study not only expressed greater blood pressure hyperreactivity to both the anticipatory and cold stressors, but also exhibited impaired recovery from stress. A previous follow-up study estimated that men with an elevated recovery blood pressure had over twice the likelihood of developing hypertension than men with efficient recovery [18]. Individuals with poor blood pressure recovery also were shown to have increased likelihood of increased blood pressure after 3 years of follow-up [19]. The importance of blood pressure recovery has only recently been appreciated [12] and provides additional support for using functional stress testing paradigms. Notably, a hypertensive response to stress was significantly more likely to persist in the arsenic-exposed men even after 10 min of recovery.

It was expected that many of our participants would reveal hypertensive blood pressure responses during cold stress. In fact, roughly half of both groups demonstrated episodic hypertension at some point over the course of stress testing. However, poor blood pressure recovery from cold stress caused a higher frequency of persistent hypertensive blood pressure responses in the exposed group. Therefore, in addition to demonstrating blood pressure hyperreactivity, the exposed group exhibited a hypertensive response profile that suggested the presence of existing masked hypertension. Masked hypertension exists when a patient's blood pressure is clinically normal but hypertensive in an ambulatory state [36]. Masked hypertension is clinically important, given its association with similar target organ damage as sustained hypertension [37].

Support for the mechanistic plausibility of the relationship between arsenic and blood pressure dysfunction can be found in studies of both animal models and humans. In animals, iAs has been observed to increase peripheral vascular resistance [38], provoke changes that increase vasoconstriction over vasorelaxation [39,40], and inhibit endothelial nitric oxide vasodilatory function [40,41]. iAs has also been demonstrated to have a direct impact on blood pressure in rats [40–42]. Recent work has demonstrated both increased blood pressure and concentric LVH in animals chronically exposed to moderate levels of iAs in drinking water [22]. In humans, the Health Effects of Arsenic Longitudinal Study (HEALS) in Bangladesh demonstrated blood pressure abnormalities associated with arsenic exposure [8]. In a study conducted in Mongolia, women who consumed moderate levels of arsenic in drinking water had significantly increased SBP and DBP [43]. Furthermore, arsenic remediation in China led to improved peripheral vascular response to cold stress, as well as general recovery of the nitric oxide/cyclic guanosine 3',5'-monophosphate system [24].

Our finding of sympathetically induced hyperreactivity in exposed individuals adds novel insight by providing a plausible mechanistic link between iAs exposure and early risk of hypertension. Integrating our findings into the current literature, it is possible that iAs (or its metabolites) may contribute to hypertension risk through increased LVH [22,23]. Shown to be sympathetically mediated [44], LVH is a risk factor for hypertension in humans [45] and has been demonstrated to be of similar magnitude in states of hypertension and masked hypertension [46]. Higher levels of cell adhesion molecules were recently associated with LVH [45]. These biomarkers are also associated with the level of iAs exposure in humans [47]. Therefore, this mechanistic hypothesis may provide future directions for physiological research into cardiovascular disease risk related to arsenic exposure.

Our study is unique in that it investigated early signs of cardiovascular risk using a physiological challenge model not often applied to environmental health exposures. There were several advantages to our controlled study that can be used to inform further physiological investigations into the mechanisms underlying the effects of iAs exposure on cardiovascular health, as well as larger epidemiological studies. We studied healthy, middle-aged men, who were homogenous in ethnicity, culture, diet, occupational, and lifestyle characteristics. Importantly, these men were longstanding residents in the target areas (20 years) known to have stable levels of iAs in drinking water [26]. No co-exposures from arsenic-related occupations existed. Exposure assessment in this study was comprehensive, including both external (water source) and internal (urine) measures of arsenic at the time of blood pressure testing. In addition, our groups were similar based on common risk factors for hypertension, including smoking, family history of hypertension, and overweight body habitus. Baseline clinical blood pressures were representative of a recent Romanian population study [29]. In short, we found exposure to be a significant predictor of both stress-induced blood pressure hyperreactivity and persistent hypertensive response to cold stress, whereas the common risk factors were not. The similarity of stress-induced blood pressure responses observed across mental and physical stressors will facilitate developing protocols to further investigate the effects of iAs exposures on early cardiovascular risk in larger populations.

Conducting the study in rural communities was necessary in order to obtain a well documented, stable, exposed population; however, this resulted in certain limitations, including unequal exposure group sizes due to participation criteria. In addition, resources were limited to the use of a standard clinical sphygmomanometer, rather than a continuous beat-to-beat blood pressure measuring device. Mental health of study participants was not formally assessed with specific interview questions; however, while depression may have a link to iAs exposure [10], it has not been demonstrated to influence the CPT response [48]. Further work is needed to examine the effects of low-to-moderate arsenic exposure on stress

responses and stress recovery in larger cohorts that include women, as well as different age and ethnic groups. Women were not selected for this proof-of-concept study because arsenic metabolism is known to vary by factors such as sex, with a more favorable metabolism occurring in women [49].

In conclusion, our study demonstrates the ability for a robust physiological challenge to discern cardiovascular dysfunction in normotensive men chronically exposed to low-to-moderate iAs in drinking water. Our findings provide novel evidence that low-to-moderate iAs exposure increases early cardiovascular risk, observed as stress-induced blood pressure hyperreactivity, poor blood pressure recovery from stress, and persistent hypertensive responses to stress in men. Exposure to iAs through drinking water is a global health problem, and functional stress testing may offer a unique strategy to assess early risk for hypertension as well as the effectiveness of health interventions in regions of elevated iAs exposure.

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Abbreviations:

ASHRAM	Arsenic Health Risk Assessment Molecular Epidemiology
CPT	cold pressor test
DMA^V	dimethylarsinic acid
iAs	inorganic arsenic
LVH	left-ventricular hypertrophy
MMA^V	monomethylarsonic acid
PMI	primary methylation index
SMI	secondary methylation index

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FIGURE 1. Blood pressure response during anticipatory stress. (a) SBP during anticipatory stress. (b) DBP during anticipatory stress (main effect * P 0.015; interaction † P <0.050).

FIGURE 2.

Blood pressure response during cold stress. (a) SBP during cold stress. (b) DBP during cold stress (main effect $*P$ 0.010; interaction $\dagger P$ 0.035).

FIGURE 3.

Percentage of hyperreactive, episodic hypertensive, and persistent hypertensive blood pressure responses. (a) Percentage of hyperreactivity in response to anticipatory and cold stress. Percentage of episodic (b) and persistent (c) hypertensive blood pressure responses during stress testing. * $P < 0.050$.

TABLE 1Description of the sample by exposure group^a

Characteristic	Unexposed (<i>n</i> = 16)	Exposed (<i>n</i> = 19)	<i>P</i> -value ^b
Age (years)	42.6 ± 6.0	43.7 ± 6.5	0.611
BMI (kg/m ²)	24.6 ± 4.7	27.7 ± 5.2	0.076
Smoking			1.000
Current nonsmoker	10 (62.5)	12 (63.2)	
Current smoker	6 (37.5)	7 (36.8)	
Alcohol			0.347
2 drinks/day	15 (93.8)	15 (79.0)	
>2 drinks/day	1 (6.3)	4 (21.1)	
Family history of hypertension			1.000
No	12 (75.0)	14 (73.7)	
Yes	4 (25.0)	5 (26.3)	

^aTable values are mean ± SD for continuous variables and *n* (column %) for categorical variables. Percentages may not sum to 100% due to rounding.

^b*P*-value is for *t*-test (continuous variables) or Fisher's exact test (categorical variables).

TABLE 2Water and urinary arsenic levels by exposure group^a

Arsenic (µg/l)	Unexposed (n = 16)	Exposed (n = 19)	P-value ^b
Water iAs	1.0 ± 0.2	40.2 ± 30.4	<0.001
Urinary iAs	0.7 ± 0.5	6.1 ± 2.4	<0.001
Urinary MMA ^V	0.9 ± 0.6	9.8 ± 4.4	<0.001
Urinary DMA ^V	4.3 ± 2.2	36.8 ± 18.9	<0.001
Primary methylation index	1.6 ± 0.9	1.6 ± 0.4	0.896
Secondary methylation index	6.1 ± 3.8	4.2 ± 2.0	0.063

DMA^V, dimethylarsinic acid; iAs, total inorganic arsenic; MMA^V, monomethylarsonic acid.

^aTable values are mean ± SD.

^bP-value is for multivariate ANOVA analysis comparing exposure groups.

TABLE 3Heart rate measurements by exposure group^a

Heart rate (b.p.m.)	Unexposed (<i>n</i> = 16)	Exposed (<i>n</i> = 19)	<i>P</i> -value ^b
Clinical	72.4 ± 6.5	71.5 ± 1.7	–
Anticipatory stress	71.6 ± 13.7	68.6 ± 14.2	>0.483
Recovery from anticipatory stress	70.4 ± 13.0	69.5 ± 12.2	>0.409
Cold stress	75.5 ± 12.7	74.5 ± 12.7	>0.181
Recovery from cold stress	69.8 ± 12.1	68.8 ± 12.6	>0.314

^aTable values are mean ± SD.^b*P*-values for repeated measures ANOVA with planned contrasts to clinical baseline for main effect of condition. Note: interaction with exposure had *P*-values of greater value. Main effect for exposure *P* = 0.629.

TABLE 4Blood pressure differences from baseline by exposure group^a

Stress testing phase		Unexposed (n = 16)	Exposed (n = 19)	P-value ^b
Anticipatory stress	SBP	11.1 ± 20.2	24.7 ± 24.8	0.086
	DBP	11.2 ± 9.5	20.0 ± 14.6	0.047
Recovery from anticipatory stress	SBP	4.3 ± 24.3	17.7 ± 25.3	0.119
	DBP	10.5 ± 13.2	20.9 ± 13.4	0.027
Cold stress	SBP	20.6 ± 23.2	38.5 ± 24.2	0.033
	DBP	23.2 ± 16.6	34.0 ± 14.7	0.049
Recovery from cold stress	SBP	1.7 ± 14.9	16.8 ± 22.3	0.028
	DBP	10.3 ± 10.7	19.6 ± 12.7	0.026

^aTable values are mean ± SD expressed in mmHg for systolic (SBP) and diastolic (DBP) blood pressures.^bP-value is for multivariate ANOVA comparing exposure status for absolute increments in SBP and DBP across stressors and recovery.