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Supplemental Material

Effect of an Arsenic Mitigation Program on Arsenic Exposure in American Indian Communities: A Cluster Randomized Controlled Trial of the Community-Led Strong Heart Water Study Program

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Table of Contents

Urinary Arsenic Analysis Standard Operating Procedure

Table S1. Baseline participant characteristics overall and by household water arsenic ($\mu\text{g/L}$) quartile for the Strong Heart Water Study randomized controlled trial.

Table S2. Baseline participant characteristics overall and by urinary arsenic ($\mu\text{g/g}$ creatinine) quartile in the Strong Heart Water Study randomized controlled trial.

Table S3. Regression of urinary total arsenic ($\mu\text{g/g}$ creatinine) at final follow-up visit comparing participants reporting exclusive arsenic-safe water use to participants reporting non-exclusive arsenic-safe water use (N=71) in the Strong Heart Water Study randomized controlled trial.

Table S4. Water source used to prepare drink and food items for participants in the Strong Heart Water Study randomized controlled trial.

Table S5. Water source used to prepare drink and food items by study arm (N=75) for participants in the Strong Heart Water Study randomized controlled trial.

Additional File- Excel Document

Supplementary File 1

Urinary Arsenic Analysis Standard Operating Procedure

The following procedures were carried out with urine samples from the Strong Heart Water Study, urine certified reference materials (CRMs) PC-U-S1909, PC-U-S1910 and PC-U-S1911 (QMEQAS - Quebec Multielement External Quality Assessment Scheme, Canada), and urine standard reference materials SRM 2669 levels I and II (Arsenic Species in Frozen Human Urine; National Institute of Standards and Technology, US). Method blanks (n=18) were treated in the same way as urine samples.

An aliquot of urine (100 μL) was transferred into an autosampler vial (PP, 700 μL), 10 μL of an aqueous hydrogen peroxide solution (30%, m/m) was added, and the mixture heated to 60 $^{\circ}\text{C}$ for 30 min. After cooling to room temperature, 390 μL of mobile phase (see following paragraphs) were added and the sample was analyzed using high-performance liquid chromatography (HPLC) coupled to elemental detection using inductively coupled plasma triple quadrupole mass spectrometry (ICPMS/MS).

Separation of individual arsenic species was performed on an Agilent 1260 Infinity-II BioInert series system using a Hamilton PRP-X100 (250 x 4.1 mm, 10 μm particles) analytical column under isocratic elution conditions applying an aqueous mobile phase containing 10 mM phosphate (ammonium phosphate dibasic; $\geq 99.0\%$, Sigma-Aldrich), 10 mM nitrate (67-70% Optima, Fisher Scientific), and 0.5% vol acetonitrile ($\geq 99.9\%$; Fisher Sci.) adjusted to pH 9.1 using aqueous ammonia solution (20-22%; Fisher Sci.). The mobile phase flow rate was set to 2 mL min^{-1} , the column was held at 30 $^{\circ}\text{C}$ during analysis, and the injection volume was 100 μL .

Detection of individual arsenic species was performed on an Agilent 8900 series ICPMS/MS system in oxygen reaction mode after HPLC separation. A modified standard ICPMS/MS set-up was employed for all experiments, consisting of a Micro Mist (glass) nebulizer, a glass double pass spray chamber, platinum/nickel sampler and skimmer cones, and a quartz plasma torch with an inner diameter of 2.5 mm. The operating ICPMS/MS parameters were as follows: radiofrequency (RF) power: 1550 W; plasma gas: 15.0 L min^{-1} ; auxiliary gas: 0.9 L min^{-1} ; RF matching: 1.70 V; sampling depth: 8.0 mm; nebulizer gas flow rate: 0.90 L min^{-1} ; makeup gas (argon) flow rate: 0.15 L min^{-1} ; spray chamber temperature: 2 $^{\circ}\text{C}$. Arsenic species were detected in oxygen reaction mode (O_2 flow rate: 20%) by monitoring the mass transitions m/z 75 \rightarrow 91 (^{75}As and $^{40}\text{Ar}^{35}\text{Cl}$), m/z 77 \rightarrow 93 (^{77}Se and $^{40}\text{Ar}^{37}\text{Cl}$, as a check for possible chloride interferences), m/z 82 \rightarrow 98 (^{82}Se , as a cross check for ^{77}Se), and m/z 53 \rightarrow 69 ($^{40}\text{Ar}^{13}\text{C}$, monitoring carbon content in plasma) at dwell times of 300, 100, 100, and 10 ms, respectively.

External calibration was performed in matrix matched mixed arsenic species standards for arsenobetaine (AB), dimethylarsinic acid (DMA), methylarsonic acid (MA), and arsenate [As(V)] in concentration ranges of 0.1-10 $\mu\text{g As L}^{-1}$ (AB and DMA) and 0.02-2 $\mu\text{g As L}^{-1}$ [MA and As(V)]. The methods' detection limits (MDLs) were calculated individually for all four arsenic species using the formula: $\text{MDL} = 3.33 \times \text{standard deviation} \times \text{sample dilution factor}$ (of 18 method blanks); the MDLs were 0.02 $\mu\text{g As L}^{-1}$ urine (AB, DMA, and MA) and 0.03 $\mu\text{g As L}^{-1}$ urine [As(V)]. The method's quantitation limits (MQLs) were calculated using the formula: $\text{MQL} = 10 \times \text{standard deviation} \times \text{sample dilution factor}$ (of 18 method blanks); the MQLs were 0.05 $\mu\text{g As L}^{-1}$ urine (AB and MA), 0.06 $\mu\text{g As L}^{-1}$ urine (DMA), and 0.08 $\mu\text{g As L}^{-1}$ urine [As(V)].

Accuracy of the method for arsenic speciation analysis was determined based on the five urinary CRMs (n=12 each) and resulted in accuracies (mean \pm s.d.) of 101 ± 12 , 100 ± 10 , 93 ± 9 , and $98 \pm 16\%$ for AB, DMA, MA, and As(V), respectively. Intraday and interday coefficients of variation (CV) were calculated based on the five certified reference urines (n=12 each) and resulted mean intraday CVs of 1.7, 1.9, 2.5, and 2.1%; and interday CVs of 2.9, 3.2, 4.0, and 3.8% for AB, DMA, MA, and As(V), respectively.

Supplementary Table S1. Baseline participant characteristics overall and by household water arsenic ($\mu\text{g/L}$) quartile for the Strong Heart Water Study randomized controlled trial.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Household water arsenic ($\mu\text{g/L}$)	6.04-11.85	11.85-16.00	16.00-21.75	21.75-210
Participants	21	20	22	21
Households	13	13	14	10
Age, years	52 (18)	51 (19)	55 (18)	59 (22)
12-17	1 (5%)	2 (10%)	0 (0%)	3 (14%)
≥ 18	20 (95%)	18 (90%)	22 (100%)	18 (86%)
Female	8 (38%)	8 (40%)	10 (45%)	13 (62%)
Education				
High school or less	9 (43%)	13 (65%)	15 (68%)	10 (48%)
More than high school	12 (57%)	7 (35%)	7 (32%)	11 (52%)
Ever smoked	17 (81%)	15 (75%)	21 (95%)	16 (76%)
BMI	30 (7)	32 (13)	28 (4)	29 (8)
Overweight (BMI ≥ 25)	15 (71%)	13 (65%)	18 (82%)	13 (62%)
Obesity (BMI ≥ 30)	10 (48%)	8 (40%)	7 (32%)	7 (33%)
Hypertension*	11 (52%)	8 (40%)	11 (50%)	11 (52%)
Systolic blood pressure	128 (23)	128 (18)	129 (19)	131 (16)
Diastolic blood pressure	75 (13)	78 (15)	79 (11)	76 (10)
Percent water use outside home	30.2 \pm 38.9	33.6 \pm 34.4	28.9 \pm 37.7	14.1 \pm 21.1
Mean \pm SD (min-max)	(0-100)	(0-100)	(0-100)	(0-70)

†Values given as n (% of total) or mean (standard deviation)

*Based on average systolic bp >130 or diastolic >80 during 3 consecutive readings

Supplementary Table S2. Baseline participant characteristics overall and by urinary arsenic ($\mu\text{g/g}$ creatinine) quartile in the Strong Heart Water Study randomized controlled trial

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Urinary arsenic range	0.21 - 7.48	7.48 - 13.42	13.42-24.39	24.39-136.42
Participants	20	21	20	23
Households	16	17	18	16
Age, years	47 (21)	48 (19)	56 (15)	64 (17)
12-17	3 (15%)	1 (5%)	1 (5%)	1 (4%)
≥ 18	17 (85%)	20 (95%)	19 (95%)	22 (96%)
Female	10 (50%)	10 (48%)	9 (45%)	10 (44%)
Education				
High school or less	13 (65%)	11 (52%)	12 (60%)	11 (49%)
More than high school	7 (35%)	10 (48%)	8 (40%)	12 (52%)
Ever smoked	14 (70%)	18 (86%)	18 (90%)	19 (83%)
BMI	33 (12)	29 (6)	29 (9)	28 (6)
Overweight (BMI ≥ 25)	15 (75%)	16 (76%)	13 (65%)	15 (55%)
Obesity (BMI ≥ 30)	10 (50%)	8 (38%)	8 (40%)	6 (26%)
Hypertension*	12 (60%)	9 (43%)	8 (40%)	12 (52%)
Systolic blood pressure	130 (20)	124 (18)	128 (20)	133 (18)
Diastolic blood pressure	79 (13)	77 (14)	74 (13)	77 (9)
Percent water use outside home				
Mean \pm SD (min-max)	39.4 \pm 41.6 (0-100)	30.2 \pm 30.3 (0-100)	18.6 \pm 34.0 (0-100)	19.3 \pm 27.8 (0-100)
Household water arsenic [§] ($\mu\text{g/L}$)	16.2 (6.9)	15.4 (6.6)	14.8 (5.2)	32.9 (47.9)

†Values given as n (% of total) or mean (standard deviation)

*Based on average systolic bp >130 or diastolic >80 during 3 consecutive readings

[§]Household water arsenic reported at the household level

Supplementary Table S3. Regression of urinary total arsenic ($\mu\text{g/g}$ creatinine) at final follow-up visit comparing participants reporting exclusive arsenic-safe water use to participants reporting non-exclusive arsenic-safe water use (N=71) in the Strong Heart Water Study randomized controlled trial

Exclusive arsenic-safe water use	Exclusive Use		Non-exclusive Use		Adjusted Linear Regression†		
	N	Urinary Arsenic GM (range)	N	Urinary Arsenic GM (range)	GM ratio	95% CI	p-value
<i>Cooking</i>	37	5.68	34	9.35	0.31	(0.11, 0.85)	0.024
<i>Drinking</i>	32	5.58	39	8.89	0.30	(0.11, 0.81)	0.017
<i>Cooking and drinking</i>	29	5.64	42	8.54	0.34	(0.12, 0.95)	0.039

Arsenic-safe source options included use of the point of use (POU) arsenic filter faucet, bottled water, or the municipal water system, and arsenic-unsafe sources included use of the kitchen faucet, bathroom faucet, and refrigerator filter or icemaker (if not reported to be connected to the POU arsenic filter faucet). Urinary arsenic ($\mu\text{g/g}$ creatinine) was defined as the sum of inorganic arsenic (iAs), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) measurements, with geometric means reported. GM indicates geometric mean. N indicates number of participants. CI indicates confidence interval. Participants without urinary arsenic data or arsenic-safe water use data excluded. Regression analyses were performed using generalized estimating equations to account for clustering at the household-level using an exchangeable correlation. Urinary arsenic is log-transformed for analysis. †Regression adjusted for sociodemographic factors (sex and age) and lifestyle factors (ever smoking and baseline BMI).

Supplementary Table S4. Water source used to prepare drink and food items for participants in the Strong Heart Water Study randomized controlled trial

Overall Study Population with Baseline and Follow-up Visits											
Consumption of items prepared using water†	Baseline (N = 75)				Follow-up (N = 71)						
	Arsenic-safe source		Arsenic-unsafe source		Arsenic-safe source			Arsenic-unsafe source			Total n*
	Bottled water	Kitchen faucet	Other‡	Total n*	Arsenic filter faucet	Bottled water	Municipal water	Kitchen faucet	Other‡		
% (n)	% (n)	% (n)	Total n*	% (n)	% (n)	% (n)	% (n)	% (n)	Total n*		
Drink Items											
Any Drink Item	17 (13)	73 (55)	8 (6)	75	72 (51)	8 (6)	4 (3)	28 (20)	10 (7)	71	
<i>Ice</i>	13 (6)	62 (29)	9 (4)	47	62 (33)	2 (1)	4 (2)	26 (14)	13 (7)	53	
<i>Tea/Coffee</i>	20 (13)	78 (50)	3 (2)	64	72 (48)	7 (5)	2 (1)	22 (15)	1 (1)	67	
<i>Juices</i>	18 (9)	78 (40)	2 (1)	51	75 (38)	8 (4)	0 (0)	18 (9)	4 (2)	51	
<i>Powdered milk</i>	15 (2)	85 (11)	8 (1)	13	60 (9)	13 (2)	20 (3)	33 (5)	0 (0)	15	
Food Items											
Any Food Item	16 (12)	76 (57)	1 (1)	75	78 (55)	5 (4)	4 (3)	25 (18)	0 (0)	71	
<i>Soup</i>	15 (10)	83 (55)	2 (1)	66	79 (50)	5 (3)	5 (3)	22 (14)	0 (0)	63	
<i>Baked goods</i>	17 (11)	81 (52)	2 (1)	64	85 (50)	5 (3)	5 (3)	19 (11)	0 (0)	59	
<i>Pasta</i>	15 (10)	85 (55)	2 (1)	65	76 (53)	6 (4)	4 (3)	23 (16)	0 (0)	70	
<i>Rice</i>	16 (9)	83 (48)	0 (0)	57	82 (55)	3 (2)	5 (3)	19 (13)	0 (0)	67	
<i>Gravy</i>	19 (11)	81 (47)	2 (1)	58	77 (48)	6 (4)	5 (3)	21 (13)	0 (0)	62	

†Detailed item descriptions: (1) ice (e.g. homemade ice), (2) juices (e.g. fruit punch, lemonade, Kool-Aid soup), (3) soup or stew (e.g. homemade or powdered), (4) baked goods (e.g. bread, muffins, pancakes, cake, cookies, waffles), (5) pasta (e.g. pasta, grains, or boiled vegetables), (6) rice (e.g. rice made with water), and (7) gravy (e.g. gravy made with water). ‡Other category includes refrigerator filter, icemaker and bathroom faucet. *Multiple sources possible per participant. n indicates number of participants. Total for specific items does not include individuals who reported no consumption of the specific item. 4 participants at follow-up did not provide water use information. 2 households were connected to the municipal water system prior to final follow-up.

Supplementary Table S5. Water source used to prepare drink and food items by study arm (N=75) for participants in the Strong Heart Water Study randomized controlled trial

mHealth & filter arm with baseline and follow-up visits (N = 47)										
Consumption of items prepared using water†	Baseline				Follow-up					
	Arsenic-safe source	Arsenic-unsafe source		Total n*	Arsenic-safe source			Arsenic-unsafe source		Total n*
	Bottled water	Kitchen faucet	Other‡		Arsenic filter faucet	Bottled water	Municipal water	Kitchen faucet	Other‡	
% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	
Drink Items										
Any Drink Item	13 (6)	87 (40)	4 (2)	46	68 (30)	7 (3)	7 (3)	27 (12)	9 (4)	44
<i>Powdered milk</i>	20 (2)	90 (9)	0 (0)	10	63 (5)	0 (0)	0 (0)	38 (3)	0 (0)	8
<i>Tea/Coffee</i>	15 (6)	93 (37)	0 (0)	40	68 (28)	5 (2)	7 (3)	24 (10)	0 (0)	41
<i>Juices</i>	15 (5)	88 (29)	0 (0)	33	70 (21)	7 (2)	3 (1)	20 (6)	3 (1)	30
<i>Ice</i>	7 (2)	68 (21)	7 (2)	31	59 (20)	0 (0)	6 (2)	21 (7)	12 (4)	34
Food Items										
Any Food Item	13 (6)	87 (40)	0 (0)	46	71 (31)	2 (1)	7 (3)	25 (11)	0 (0)	44
<i>Baked goods</i>	16 (7)	86 (38)	0 (0)	44	74 (26)	3 (1)	9 (3)	17 (6)	0 (0)	35
<i>Gravy</i>	15 (6)	87 (34)	0 (0)	39	74 (29)	3 (1)	8 (3)	21 (8)	0 (0)	39
<i>Pasta</i>	14 (6)	90 (38)	0 (0)	42	74 (32)	2 (1)	7 (3)	21 (9)	0 (0)	43
<i>Rice</i>	14 (5)	89 (33)	0 (0)	37	76 (32)	2 (1)	7 (3)	19 (8)	0 (0)	42
<i>Soup</i>	10 (4)	93 (39)	0 (0)	42	74 (28)	3 (1)	8 (3)	24 (9)	0 (0)	38

Supplementary Table S5 cont. Water source used to prepare drink and food items by study arm (N=75) for participants in the Strong Heart Water Study randomized controlled trial

Intensive arm with baseline and follow-up visits (N = 28)											
Consumption of items prepared using water†	Arsenic-safe source		Arsenic-unsafe source		Total n*	Follow-up					Total n*
	Bottled water	%	Kitchen faucet	Other‡		Arsenic-safe source			Arsenic-unsafe source		
						Arsenic filter faucet	Bottled water	Municipal water	Kitchen faucet	Other‡	
% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)		
Drink Items											
Any Drink Item	25 (7)		54 (15)	14 (4)	28	78 (21)	11 (3)	0 (0)	30 (8)	11 (3)	27
<i>Powdered milk</i>	0 (0)		67 (2)	33 (1)	3	57 (4)	29 (2)	0 (0)	29 (2)	0 (0)	7
<i>Tea/Coffee</i>	29 (7)		54 (13)	8 (2)	24	77 (20)	12 (3)	0 (0)	19 (5)	4 (1)	26
<i>Juices</i>	18 (4)		39 (11)	4 (1)	28	81 (17)	10 (2)	0 (0)	14 (3)	5 (1)	21
<i>Ice</i>	25 (4)		50 (8)	13 (2)	16	68 (13)	5 (1)	0 (0)	37 (7)	16 (3)	19
Food Items											
Any Food Item	21 (6)		61 (17)	4 (1)	28	85 (23)	11 (3)	0 (0)	26 (7)	0 (0)	27
<i>Baked goods</i>	20 (4)		70 (14)	5 (1)	20	86 (21)	8 (2)	0 (0)	21 (5)	0 (0)	24
<i>Gravy</i>	26 (5)		68 (13)	5 (1)	19	82 (19)	13 (3)	0 (0)	22 (5)	0 (0)	23
<i>Pasta</i>	17 (4)		74 (17)	4 (1)	23	78 (21)	11 (3)	0 (0)	26 (7)	0 (0)	27
<i>Rice</i>	20 (4)		75 (15)	0 (0)	20	92 (23)	4 (1)	0 (0)	20 (5)	0 (0)	25
<i>Soup</i>	25 (6)		67 (16)	4 (1)	24	88 (22)	8 (2)	0 (0)	20 (5)	0 (0)	25

†Detailed item descriptions: (1) ice (e.g. homemade ice), (2) juices (e.g. fruit punch, lemonade, Kool-Aid soup), (3) soup or stew (e.g. homemade or powdered), (4) baked goods (e.g. bread, muffins, pancakes, cake, cookies, waffles), (5) pasta (e.g. pasta, grains, or boiled vegetables), (6) rice (e.g. rice made with water), and (7) gravy (e.g. gravy made with water). ‡Other category includes refrigerator filter, icemaker and bathroom faucet. *Multiple sources possible per participant. n indicates number of participants. Total for specific items does not include individuals who reported no consumption of the specific item. 4 participants at follow-up did not provide water use information. One participant with water usage information did not provide urinary arsenic information. 2 households were connected to the municipal water system prior to final follow-up.