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Supplemental Material

Drinking Water Salinity and Raised Blood Pressure: Evidence from a Cohort Study in Coastal Bangladesh

Pauline F.D. Scheelbeek, Muhammad A.H. Chowdhury, Andy Haines, Dewan S. Alam, Mohammad A. Hoque, Adrian P. Butler, Aneire E. Khan, Sontosh K. Mojumder, Marta A.G. Blangiardo, Paul Elliott, and Paolo Vineis

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Confounders and effect modifiers

Physical activity was determined by job-related physical activity and any additional self-reported physical activity that the respondents carried out in their leisure time. Respondents were asked how many hours they worked per day, in what type of activities they were involved and whether it changed a lot over time (i.e. whether it was a day-to-day or unusual activity). For each activity type, energy use per hour was estimated based on the compilation of energy expenditures by Vaz *et al* 2005 [1]. For each reported type of activity a "match" was sought in the tables compiled by Vaz *et al*; it was attempted to match mostly on tables from countries with similar climatological conditions (India, Burma, and other areas in Bangladesh) and where possible use figures based farmer communities. Some inaccuracies were expected, as studies were done among participants in other settings. Therefore, each activity was categorised as low-, medium or high intensity (cut-off points: <150 kcal/h; 150 – 300 kcal; >300kcal) with an allocated activity score of 1, 2, and 3 respectively. Each of the expenditure scores were than multiplied by the number of hours and minutes self-reported execution of these activities to calculate the total physical activity score. As also some inaccuracy was expected in the number of hours reported, final scores were grouped into 4 categories, classifying the participants as non-, low-, medium or highly active. **Weight** was measured with an analogue scale (Yamasa TY6). The scale was calibrated prior to the baseline and again prior to follow-up 1. Participants were asked to remove any (heavy) coats or jumpers. Weight was rounded to the nearest 0.5 kilogram. **Height** was measured with an aluminium tape-measure. The data collectors were instructed to find the combination of a flat floor and a straight wall to be able to accurately measure height. Measurements were rounded to the nearest 0.5 cm. **Upper arm circumference** was measured with a measuring tape. Participants were asked to remove any thick coats or jumpers if they could not be rolled up enough. Upper arm circumference was rounded to the nearest 0.1 cm. Anthropometric data were obtained only at baseline. **Weather data** were obtained from the Bangladesh Meteorological Institute on a daily basis for the entire study period, including the two weeks prior to the first baseline measurements. All reported **underlying diseases** were confirmed with the administrative books of the Health Assistants (HAs) themselves – if it did not appear in their books it was up to the judgement of the HA to declare the reported diseases as plausible or reliable. **Socio-economic status** was determined by collecting data on land ownership, type of house and roofing, as well as ownership of certain goods, such as a TV, motor cycle and bicycle, which were later used for a principal component analysis per location to determine for each participant whether they were from a relatively high, an intermediate or lower socio-economic class. Food history data for 3 days prior to the interview day were taken to estimate **dietary salt intake**. Furthermore, food samples were taken from 27 locations (2x12 households and 3 restaurants) for the 16 main dishes consumed in the study and analysed in the Nutrition and Food Science Laboratory from the Dhaka University. All data collectors were instructed to find two samples of each of the items on the list from participant or non-participant households in their study site. Convenience sampling was used: just before lunch time (which varied by Upazilla), families around the household

that was last interviewed were asked if they had food ready, and whether they agreed to the data collectors measuring portion size and taking a sample of each food item/dish. Subsequently neighbours were asked if they had prepared other items from the 16 "main dishes" list. Due to time constraints not all items were collected twice for each study site. Also, 7 samples were obtained from restaurants. Results were used to assign an estimated sodium content to each reported dish in the participants' dietary recall data. The list of 16 main dishes was also to simplify the recording of dietary histories. **Added salt** was reported in "pinches" per meal. A pinch was considered as 0.25 g of salt (0.1 g sodium). **GPS coordinates** were measured with a handheld GPS device (Garmin eTrex 10). Minimum accuracy of 3 meters was observed. All coordinates were documented in the WGS-1984 format (hd.ddddd).

Sample collection

Urine samples

To obtain spot urine samples, participants were asked to collect urine in a small sample pot. They were instructed to fill the sample pot up to the indicated line. Sample pots were labelled prior to sample taking and firmly sealed immediately after the participant returned the pot, and placed in an ice-box. Participants selected for the 24h urine collection were asked to collect their urine for 24 hours on the day prior to the interview. In a pilot study – conducted prior to the cohort study – participants were asked to discard the morning urine, however this was often misunderstood/ not correctly practised and often two morning samples were collected in a 24h period. Therefore protocols were changed: participants collected all urine from the morning onwards, and were asked to completely empty their bladder before they went to sleep in the evening. The importance of the completeness and proper collection was strongly emphasized by the data collectors. Participants received a cup and 24 hour container as well as a polystyrene box, to keep the 24-h urine container cool and avoid spillage. Samples were collected the following day by the health assistants when they came back for the interview.

Food samples

Food samples were taken just before lunch time, when families finished their cooking process. At each sample point, family members were asked to serve themselves a plate, a bowl, big spoon, etc. of the dish they prepared. This was then weighed on a digital kitchen scale (Topwe, sx-7001). Subsequently a small sample (± 5 grams) of each dish was collected in a clean plastic box and placed in an ice-box. An average weight/volume was assigned to each unit size - bowl, plate, big spoon, small spoon and piece of the 16 main dishes. Also, an estimated sodium content was determined for each dish.

Transport and analysis of samples

Water samples

After collection, water samples were transported to the study office in Dacope, where they were kept at room temperature in a polystyrene box. In the same box they were transported to the laboratory in the Department of Geology, Dhaka University, where they were analysed.

Sodium and potassium concentrations were measured using the Atomic Absorption Flame Photometry (direct aspiration) method with Air-Acetylene (oxidizing) flame.

Urine Samples

After collection of the urine samples, they were immediately transported in an ice-box (approximately 10°C) to the study centre in Dacope and stored at 4°C. A laboratory technician homogenised the 24h-specimen using a glass rod, and measured and recorded the total volume. He kept aside 10 ml of each sample for further analyses. From there the specimens were transported in a cool-box (approximately 4°C) to the clinical biochemistry laboratory of the International Center for Diarrhoeal Disease Research Bangladesh (icddr,b) for analysis. Urinary sodium and potassium were measured by indirect Ion Selective Electrode method (ISE). The lab used an automated biochemistry analyser (Beckman Coulter AU-680), which automatically dilutes the sample and potentiometrically determines the ion-activity of K^+, Na^+ and Cl^-. Individual 24-hr sodium excretion values were calculated as the product of concentrations in urine and the total urine volumes, measured in millimoles per day (mmol/d). Spot urine samples were collected after each interview and the same procedures were followed for analysis of urinary sodium and potassium concentrations.

Food Samples

Food samples were all collected on the same day and transported within 12h in an icebox (approximately 10°C) to the Nutrition and Food Science Laboratory at the University of Dhaka. On arrival they were directly stored in a freezer at -20°C. All samples were analysed in the following week using photoelectric flame photometry.

Intra-Cluster Correlation Coefficient

The intra-cluster correlation coefficient (ICC) for the selected sites was estimated based on a previously conducted case control study [2]. Blood pressure for 534 women of 24 villages over 9 unions were analysed and within and between village variations in BP values were calculated. A description of the villages and the protocols for BP measurements are described by Khan et al [2]. There were three new villages included in this study that were not part of the previous study, hence could not yet be included in the ICC calculations as no (representative) BP data were available. They were however situated in the same unions as some other villages. ICC of villages and unions showed very similar results: ICC=0.066 and 0.064 respectively. Therefore, an ICC of 0.065 was used for power calculations. It should be noted that these calculations were based on data on pregnant women and may under- or overestimate the ICC for non-pregnant adults.

Standard deviations of previously collected blood pressure means were used (9.2 and 6.1 mmHg – for SBP and DBP respectively) together with the expected changes in blood pressure, and calculated ICC to estimate power to detect a change for various significance levels.

Results Generalised Linear Mixed Models

The dNa group had the highest proportion of Hindu and the lowest proportion of people of low socioeconomic status compared with the other two groups, but there were no differences in job related physical activity or dietary salt intake between groups (Table S1).

In the between-year comparison, the median increase of sodium in the iNa group was 363 mg/l (IQR 288/1023 mg/l), compared to the control group. The median decrease in the same period for those in the dNa group was 248 mg/l (IQR -368/-223). In the controls sodium levels changed marginally (-27 mg/l [-118/-1]). In the within dry-season comparison the corresponding numbers were 524 (271/748), -308 (-690/-307) and 30 (4/108) respectively (Table S2).

Data were analysed using GLMMs. With adjustment for multiple potential confounders (Model 3, see Methods main article), compared to the control group, systolic BP of individuals in the dNa group dropped on average by 8.61 (–12.74 /-4.91) mm Hg (Figure S2.1), and for those in the iNa group, it rose on average by 8.48 (4.21/12.74) mm Hg. Similar patterns for diastolic BP changes were found: for those in the dNa group, compared to the controls, diastolic pressure dropped on average by 3.19 (-5.96/-0.41) mm Hg, while in the increased sodium group it rose by 7.05 (3.72/10.38) mm Hg. Also, for the within-dry season comparisons, increasing and decreasing salinity levels were associated with significant changes in systolic and diastolic BP (Figure S5).

These associations remained significant when sensitivity analysis was performed excluding participants who switched between dNa, iNa and the control group between measurements. Associations did not alter significantly when using water sodium consumption estimates based on average water intake instead of person specific (self-reported) water intake in the models.

Table S1: Baseline characteristics participants

	dNa (n=114)	Controls (n=330)	iNa (n=63)	All	P-value
Age *(median)*	38	37	38.5	38	
Male sex (%)	52.6	45.8	49.2	47.7	
Hours of work per day *(median)*	7	8	7	8	
Physical activity though job					
Light/sedentary work (%)	14.3	17.4	8.3	15.5	
Moderately heavy workload (%)	46.9	49.3	46.7	48.5	
Heavy workload (%)	38.8	33.3	45.0	36.1	
Body Mass Index *(mean)*	21.0	21.7	21.1	21.4	
Smoking					
Never smoked (%)	73.7	74.0	69.8	73.4	
Former smoker (%)	3.5	7.0	6.4	6.1	
Current smoker (%)	22.8	19.1	23.8	20.5	
Marital Status					
Married (%)	84.2	87.6	85.7	86.6	
Single (%)	9.7	5.8	7.9	6.9	
Separated/Widow(%)	6.1	6.7	6.4	6.5	
Religion					
Muslim (%)	28.1	42.9	31.8	38.1	0.011*
Hindu (%)	71.9	57.1	68.3	61.9	
Size of Household *(mean)*	4.2	4.3	4.3	4.3	
Education					
No education/illiterate (%)	22.8	24.2	14.3	22.7	
Primary school (%)	28.1	31.5	47.6	32.7	
Secondary school or higher (%)	49.1	44.2	38.1	44.6	
Socio-economic status					
Lowest Tertile (%)	22.8	37.9	44.4	35.3	<0.001*
Intermediate Tertile (%)	49.1	27.0	27.0	32.0	
Highest Tertile (%)	28.1	35.2	28.6	32.7	
Salt intake per adult family member *(g/ month Na^+Cl^- [mean])†*	123	121	117	121	

dNa Decreased sodium group (sodium concentration decreased between measurement points)
iNa Increased sodium group (sodium concentration increased between measurement points)
* Pearson Chi-square test
† Based on total salt used by the family per month / number of adult family members

Table S2: Median drinking water sodium concentration differences between measurement periods for each comparison group

Sodium Group	Between-year comparison		Within-year comparison	
	Median difference in sodium concentration (mg/l)*	Interquartile range (mg/l)	Median difference in sodium concentration (mg/l)**	Interquartile range (mg/l)
"Controls"	-27	-118 to -1	30	4 to 108
Increased sodium group (iNa)	363	288 to 1023	524	271 to 748
Decreased sodium group (dNa)	-248	-368 to -223	-308	-690 to -307

* between measurement point 1 and 2
** between measurement point 2 and 3

Figure S1 – Map of the study area (Khulna and sub-districts Paikghaccha, Dacope and Batiaghata)

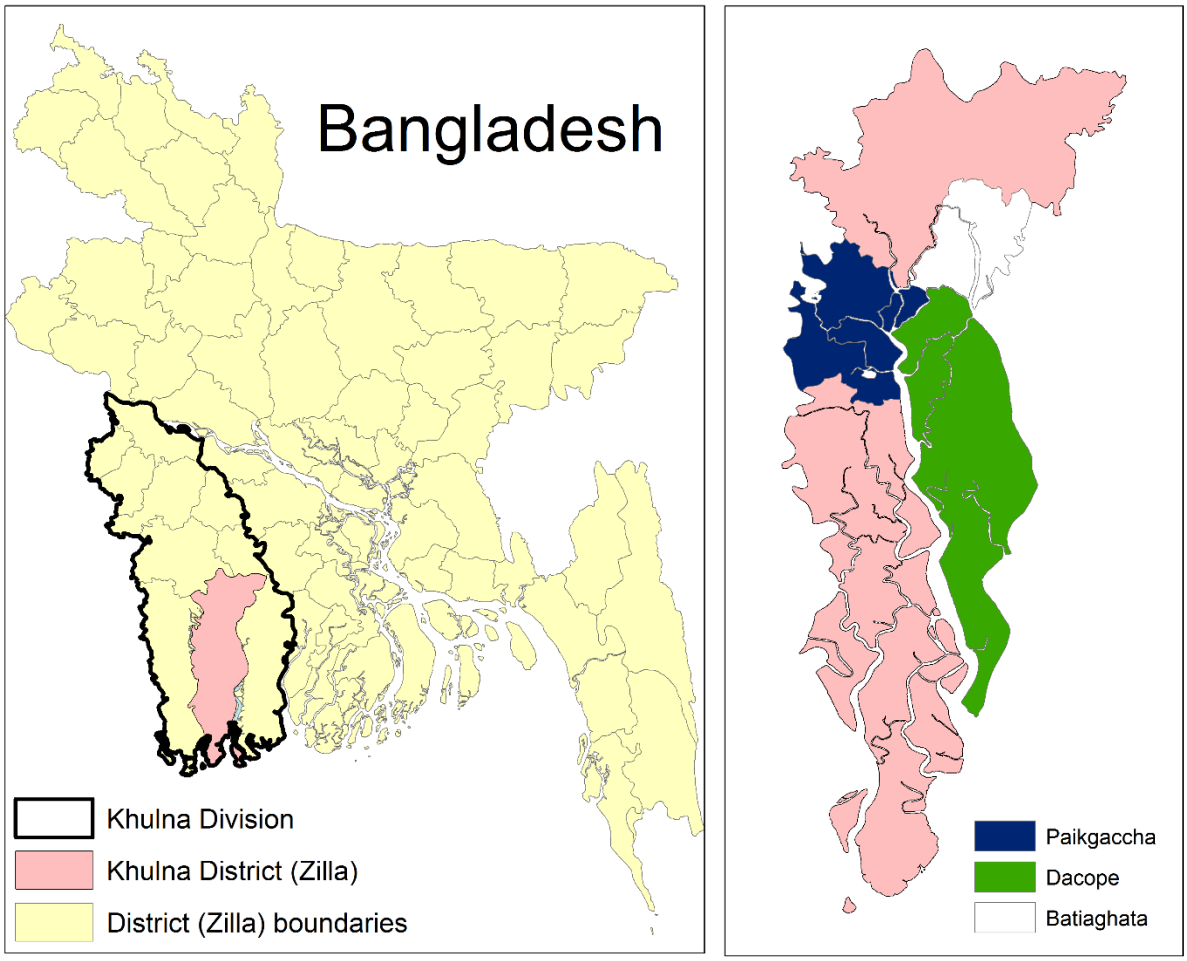


Figure S2 - Schematic representation of the study design

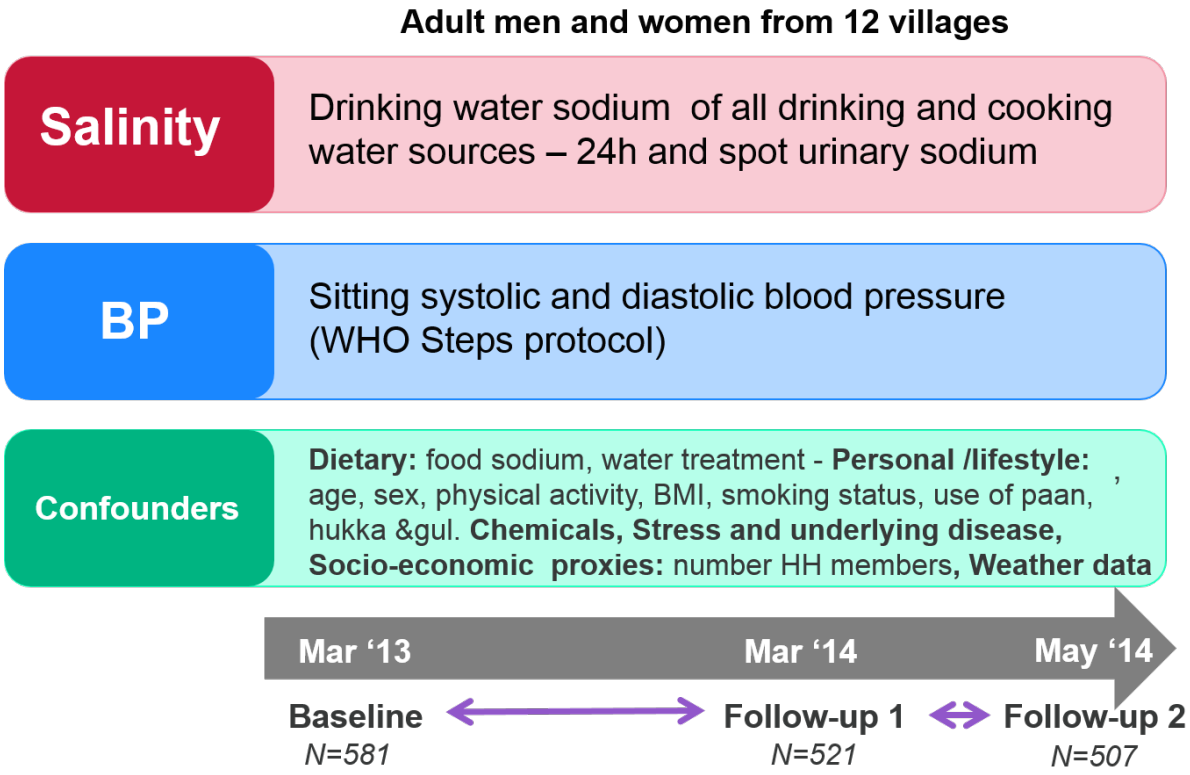


Figure S3 – Criteria for inclusion of villages and families into the scheme.

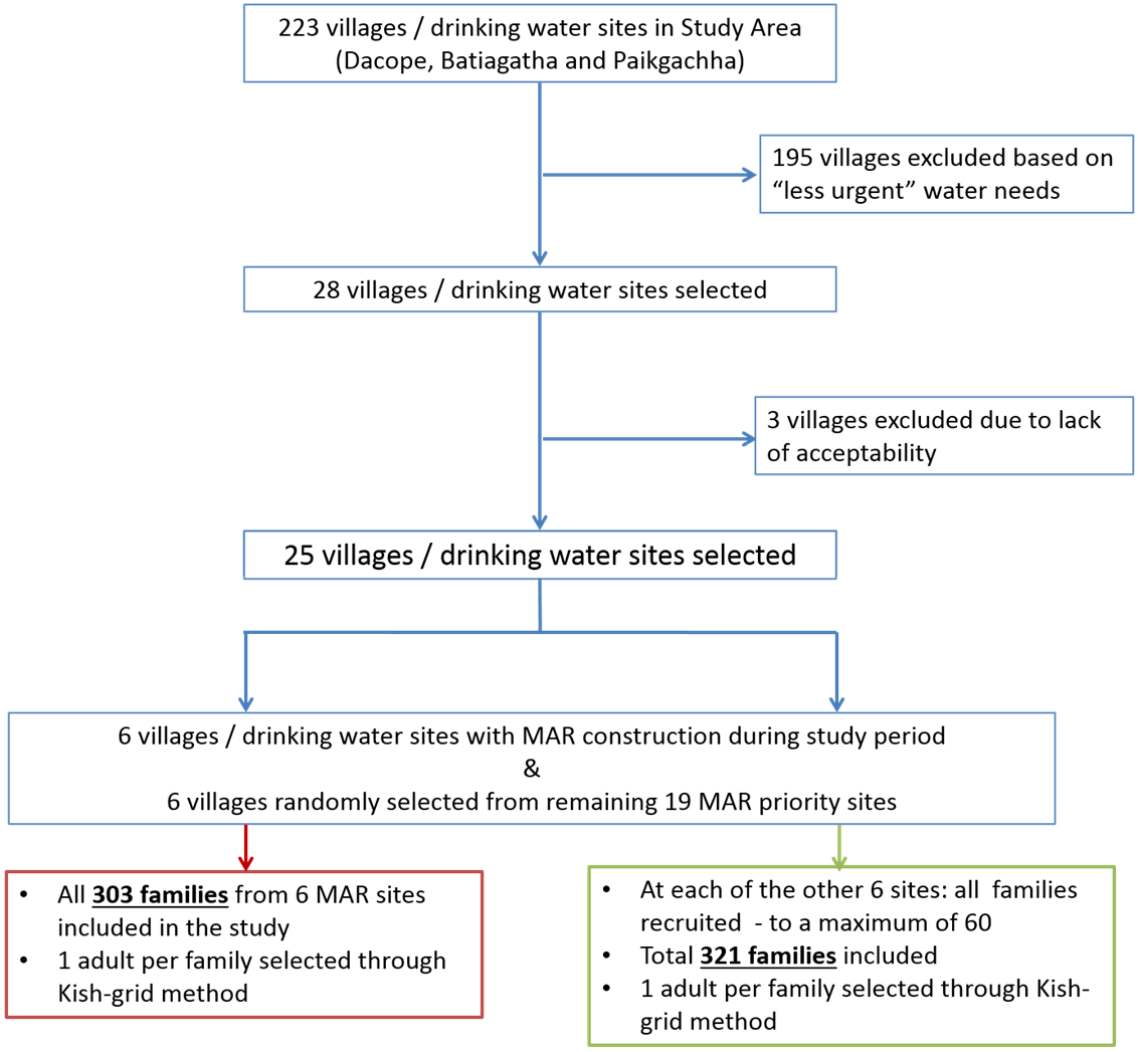


Figure S4 - Participant flow diagram for baseline and follow-up data collection periods

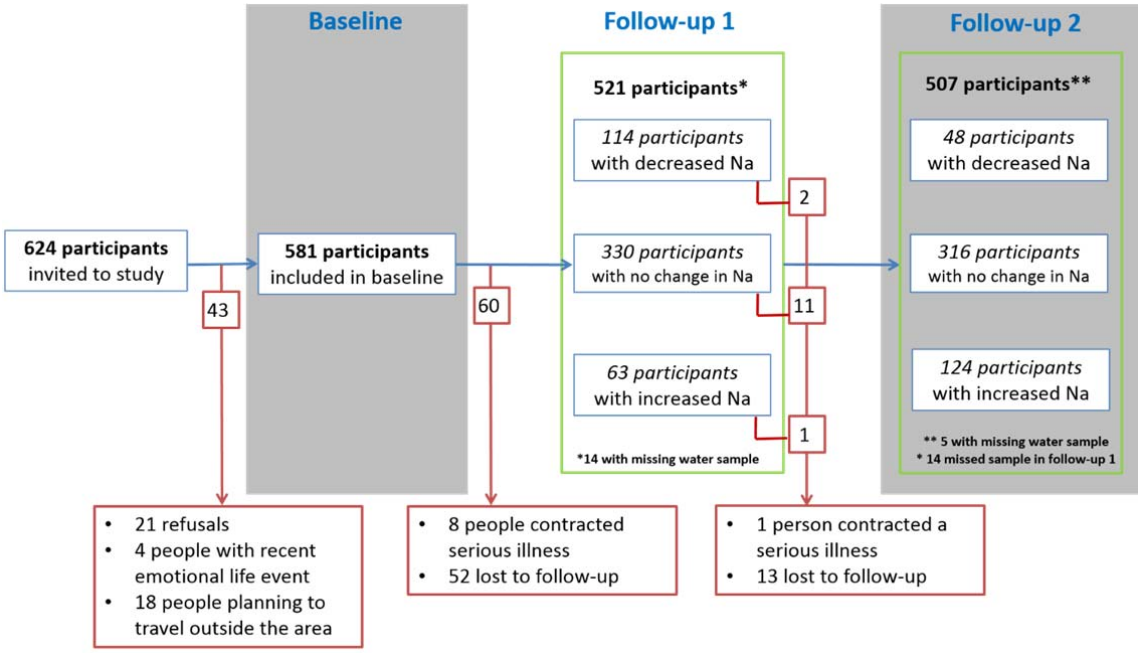
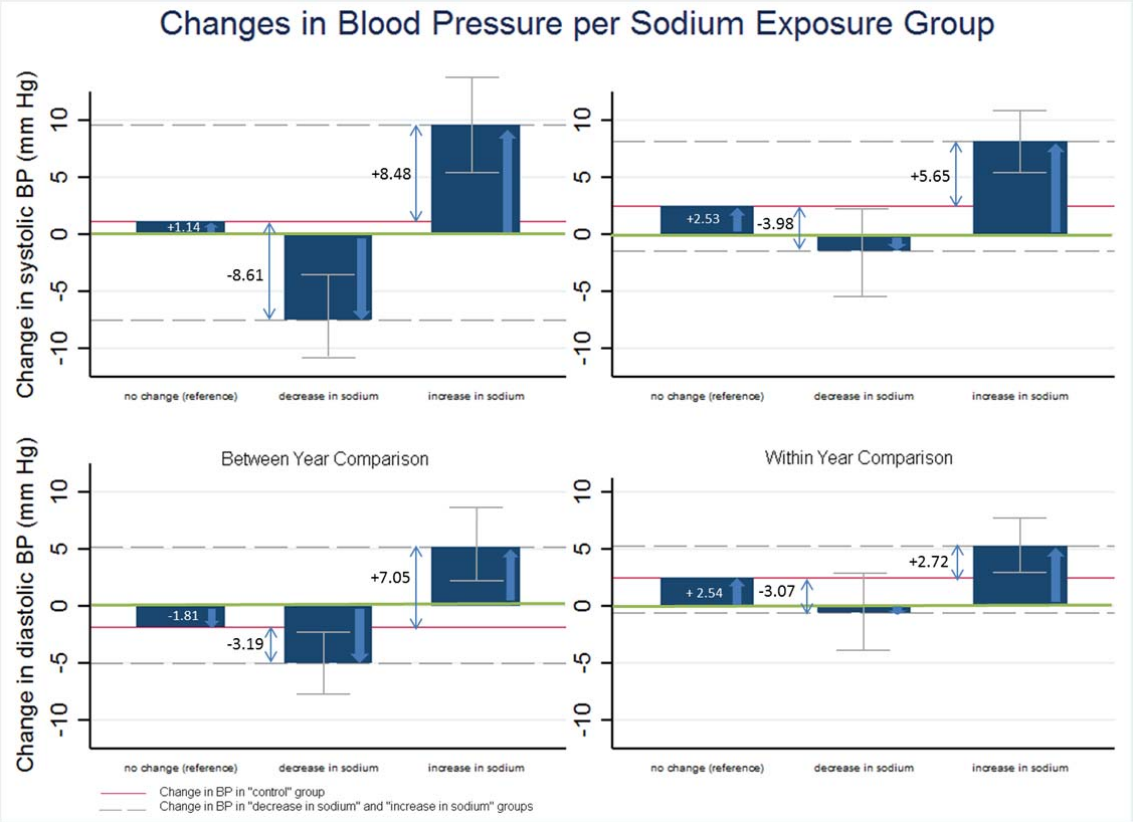


Figure S5 Changes in blood pressure per sodium exposure group



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