

Full title: Estimating population salt intake in India using spot urine samples

Short title: Estimating salt intake in India

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ABSTRACT

Objective: To compare estimates of mean population salt intake in North and South India derived from spot urine samples versus 24-hour urine collections.

Methods: In a cross-sectional survey, participants were sampled from slum, urban and rural communities in North India and in South India. Participants provided 24-hour urine collections, and random morning spot urine samples. Salt intake was estimated from the spot urine samples using a series of established estimating equations. Salt intake data from the 24-hour urine collections and spot urine equations were weighted to provide estimates of salt intake for Delhi and Haryana, and Andhra Pradesh.

Results: A total of 957 individuals provided a complete 24-hour urine collection and a spot urine sample. Weighted mean salt intake based on the 24-hour urine collection, was 8.59g/day (95% CI 7.73–9.45) and 9.46g/day (8.95–9.96) in Delhi and Haryana, and Andhra Pradesh, respectively. Corresponding estimates based on the Tanaka equation [9.04g/day (8.63–9.45) and 9.79g/day (9.62–9.96) for Delhi and Haryana, and Andhra Pradesh, respectively], the Mage equation [8.80g/day (7.67–9.94) and 10.19g/day (9.59–10.79)], the INTERSALT equation [7.99g/day (7.61–8.37) and 8.64g/day (8.04–9.23)] and the INTERSALT equation with potassium [8.13g/day (7.74–8.52) and 8.81g/day (8.16–9.46)] were all within 1g/day of the estimate based upon 24-hour collections. For the Toft equation estimates were 1-2g/day higher [9.94g/day (9.24–10.64) and 10.69g/day (9.44–11.93)] and for the Kawasaki equation they were 3-4g/day higher [12.14g/day (11.30–12.97) and 13.64g/day (13.15–14.12)], although the spot urine samples were not second morning voids as used in the original validation study.

Conclusion: In urban and rural areas in North and South India, most spot urine based equations provided reasonable estimates of mean population salt intake.

Condensed abstract

Methods for estimating salt intake based upon gold standard 24-hour urine collections are onerous. Alternative approaches based upon spot urine samples may be a viable alternative for estimation of mean population values. This study showed that in populations from North and South India, spot urine estimates of salt intake based on the Tanaka, Mage and INTERSALT equations were within 1g/day of estimates from 24-hour urine samples. For the Toft equation, the estimates were 1-2g/day higher than salt intake measured using 24-hour urine collections. The Kawasaki equation overestimated salt intake by 3-4g/day compared with 24-hour urine samples.

Keywords

Salt, sodium, 24-hour urine sample, spot urine sample, India

INTRODUCTION

In India, 27% of all deaths are attributable to cardiovascular disease, making it the leading cause of mortality [1]. Approximately one-quarter of these deaths are due to raised blood pressure. Excess salt consumption is a well-established cause of high blood pressure [2]. A recent meta-analysis showed that salt consumption in India is close to double the World Health Organization's recommended maximum of 5g/day [3]. Population salt reduction is projected to be one of the most cost-effective strategies to reduce rates of premature death and disability due to high blood pressure and vascular disease [4]. For this reason, all member states of the World Health Organization have agreed to a global target to reduce mean population salt intake by 30% by 2025 [5].

To measure and monitor population salt intake requires methods that are easy to administer, low cost and reliable. Collection of 24-hour urine samples is currently the accepted best method for measurement of population salt intake [6]. However, this method has a number of limitations, which include high participant burden, low-response rates and high rates of incomplete sample collection [7]. In addition, there are significant costs associated with the collection of 24-hour urine samples, which limits their use particularly in resource-poor settings [8].

There is some evidence to suggest that the collection of spot urine samples may be an alternative method that could be used to estimate mean population salt intake [9]. The established spot urine equations were developed in white, non-Hispanic black and

Japanese populations and there is likely to be heterogeneity in the utility of spot urine equations based on the geographic location, race and ethnicity of the population studied [8]. To date, there has been no substantive investigation of whether methods based on spot urine samples can be used to estimate population salt intake in India. An analysis from the Prospective Urban Rural Epidemiological Study examined the agreement between salt intake measured using 24-hour urine collections and spot urine based methods but included only a small number of individuals (n=87) from India and did not report India-specific findings [10].

The aims of these analyses were to calculate mean population salt intake in North and South India based upon 24-hour urine collections and established spot urine-based estimating equations, and to compare the differences.

METHODS

This manuscript comprises a secondary analysis of data collected as part of a cross-sectional survey conducted in India between February and June 2014. The methods and results for this study have been previously published [11, 12]. The study was approved by the Indian Health Ministry's Steering Committee and the Human Research Ethics Committees of the Centre for Chronic Disease Control, New Delhi and the University of Sydney, Australia. Written informed consent was obtained from all participants.

Participant selection and recruitment

Participant selection and recruitment have previously been reported [11] [12]. Briefly, participants were sampled from slum, urban and rural areas in North India (Delhi and Faridabad, Haryana) and in South India (Hyderabad, Telangana and West Godavari, Andhra Pradesh). Recruitment was stratified by sex, age and area (slum, urban and rural) and limited to one person per household. In Delhi and Haryana census enumeration blocks for urban areas and villages (for rural) were sampled at random. In Andhra Pradesh, the census enumeration blocks and villages were selected to be broadly representative of those in the State using a purposive process. In both states, a census list including information about the age and sex of residents was compiled for selected census enumeration blocks and villages and a random sample of the population were invited to participate until enrollment numbers in each stratum were filled.

Urine collection

Participants were provided with a 24-hour urine collection kit including a 50 ml container to collect a spot urine sample, and written and verbal instructions for completing the collections. A urine collection day was agreed and a reminder call was placed to the participant the evening before their scheduled starting date. On the morning of the collection day, upon rising, participants were asked to discard the first void of the day and record the date and time as the starting point for the urine collection period. They were instructed to collect all subsequent urine voids over the next 24-hour period. During the morning of the 24-hour collection, participants collected a spot urine sample into the 50 ml container provided. If the participant

reported a duration of collection of fewer than 24 hours, more than one void missed, or more than one episode of substantial spillage of a void, the participant was offered the option to redo the 24-hour collection. Urine samples were collected by field researchers on the day of completion and transferred to a local laboratory where the volume of both the spot and 24-hour urine collections were measured and aliquots were drawn for assay. Aliquots were stored locally at -20°C and were transferred to a central laboratory at the Centre for Chronic Disease Control in Delhi for analysis and the remainder of the urine was discarded. Urinary sodium and potassium were determined using the ion selective electrode method on electrolyte analyzer (XI-921) and reagents from Caretium, Shenzhen, China. Urinary creatinine was measured by the Jaffe method using an autoanalyzer (C 311) with reagents from Roche Diagnostics, Switzerland. Urinary control from RANDOX was used as internal quality control.

Estimation of salt intake from 24-hour urine samples

For each individual, the volume and concentration data for the spot and 24-hour collection was combined to derive the 24-hour sodium excretion estimate. The 24-hour sodium excretion value (mmol/day) was calculated as the concentration of sodium in the urine (mmol/L) multiplied by the urinary volume (L/day). If participants reported that the collection time was more or less than 24-hours, a time adjustment factor to standardize to a 24-hour period was applied (e.g. if urine was collected for 23 hours the sodium value (mmol/day) would have been multiplied by 1.04 ($24/23$)) to derive the 24-hour value. The conversion from sodium (mmol/day) to sodium (mg/day) was made by multiplying by 23, and the conversion from sodium (g/day) to salt (g/day) was made by multiplying the sodium value by 2.54. The 24-hour urine samples were

considered incomplete and excluded from all analyses if any of the following occurred: 1) the total 24-hour urinary volume was <500 ml; 2) the estimated daily urinary creatinine excretion was <6 mmol for men or <4 mmol for women; 3) the reported duration of collection was <24-hours; 4) more than one void was reported as missed; or 5) there was more than one episode of significant spillage of a void.

Estimation of salt intake from spot urine samples

Salt intake was estimated from spot urine samples using a series of established estimation equations. The equations used were: Tanaka [13], Kawasaki [14], Toft [15], Mage [16, 17] and INTERSALT with and without potassium [18] (Supplementary Table 1). These equations use the concentration of sodium and creatinine in the spot urine sample, as well as age, weight and height (or body mass index). In addition, the Kawasaki, Mage, Toft and INTERSALT methods have a separate equation for each sex and Mage includes a term for African American race. With the exception of the INTERSALT equations, these equations are based on the ratio of sodium to creatinine in the spot urine sample and include an estimate of 24-hour creatinine excretion to extrapolate the spot sodium concentration to a 24-hour urine value. The INTERSALT equations use spot sodium and spot creatinine concentration, age, body mass index and sex with or without spot potassium concentration to derive 24-hour salt intake.

Statistical methods

Salt intake values that were calculated from spot urine based equations and were greater than three standard deviations above or below the mean were excluded from all analyses. Salt intake data from the 24-hour urine collections and spot urine equations were weighted to provide estimates of salt intake for each region (Delhi and

Haryana, and Andhra Pradesh). The weighting procedure accounted for the strata and clustering in the study design. Sampling weights were developed from the probability of selection of the site and the number in each household, and these were then weighted to the reported population structure of Delhi and Haryana, and Andhra Pradesh, respectively. Paired samples t-tests were used to compare mean population salt intake estimated by the 24-hour urine collections and spot urine equation based methods. To determine the agreement between individual-level salt intake estimated from 24-hour collections and methods based on spot urine collections, Bland-Altman plots were used [19]. The difference between the methods was plotted against the mean of the two methods. A regression line was fitted to these data to examine whether the difference between the methods was proportional to the level of salt intake. The difference between the methods was proportional to the level of salt intake so limits of agreement based on the Regression Method were also plotted on the Bland Altman plots [20]. Two-way mixed effects model was used to determine the intraclass correlation coefficient for salt intake estimated by 24-hour urine collection and each spot urine equation. Data are presented as mean (95% confidence interval) unless otherwise specified. Statistical analyses were conducted using STATA 13.1 for windows (StataCorp. 2013 Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

RESULTS

There were 637 (89%) individuals from Delhi and Haryana and 758 (90%) individuals from Andhra Pradesh that provided 24-hour urine samples that met the sample

collection criteria. After exclusion of suspected incomplete samples based upon volumes and assays of creatinine, there were 957 (Delhi and Haryana n= 417; Andhra Pradesh n=540) that remained and that are included in these analyses (Table 1). Spot urine samples were available for all. Based upon the availability of covariates and after exclusion of outliers, a total of 946 spot urine sample were included in analyses based on the Kawasaki equation, 949 for the Tanaka equation, 945 for the Mage equation, 947 for the Toft equation, 952 for the INTERSALT equation and 953 for the INTERSALT equation with potassium.

Estimated salt intake

Based on the 24-hour urine collection, salt intake was 8.59g/day (95% CI 7.73 – 9.45) in Delhi and Haryana, and 9.46g/day (8.95 – 9.96) in Andhra Pradesh (Figure 1). For Delhi and Haryana, the corresponding salt intake estimates from spot urine samples were 12.14g/day (11.30 – 12.97) using the Kawasaki equation, 9.04g/day (8.63 – 9.45) using the Tanaka equation, 8.80g/day (7.67 – 9.94) using the Mage equation, 9.94g/day (9.24 – 10.64) using the Toft equation, 7.99g/day (7.61- 8.37) using the INTERSALT equation, and 8.13g/day (7.74 – 8.52) using the INTERSALT equation with potassium. Spot urine based salt intake estimates for Andhra Pradesh were 13.64g/day (13.15 – 14.12) using the Kawasaki equation, 9.79g/day (9.62 – 9.96) using the Tanaka equation, 10.19g/day (9.59 – 10.79) using the Mage equation, 10.69g/day (9.44 – 11.93) using the Toft equation, 8.64g/day (8.04 – 9.23) using the INTERSALT equation, and 8.81g/day (8.16 – 9.46) using the INTERSALT equation with potassium.

The sodium to potassium ratio in the 24-hour urine collections was 5.6 (5.34 – 5.76) compared to 5.81 (5.63 – 5.99) in the spot urine samples ($p=0.07$).

Agreement between methods of estimating salt intake

For estimation of mean population values, using all the data combined, compared with estimates from 24-hour urine collections, mean salt intake was substantially over-estimated by the Kawasaki equation (3.83g/day; 3.55 – 4.12; $p<0.001$), moderately overestimated by the Toft equation (1.43g/day; 1.17 – 1.70; $p<0.001$) and slightly overestimated by the Tanaka equation (0.61g/day; 0.35 – 0.87; $p<0.001$). Conversely, salt intake was slightly under-estimated by both the INTERSALT equation (0.56g/day; -0.82 – -0.31; $p<0.001$) and the INTERSALT equation with potassium (0.42g/day; -0.68 – -0.16; $p=0.002$). For the Mage equation, there was no difference detected (0.11g/day; -0.26 – 0.48; $p=0.55$), (Figure 2).

For all the estimating equations, the difference in estimated salt intake between spot and 24-hour collections for individuals was proportional to the level of salt intake. Apart from the Mage equation, compared to estimates based on 24-hour urine samples, spot urine estimates were higher at lower levels of salt intake and lower at higher intake levels. The intraclass correlation coefficients ranged from 0.25 for the Toft and INTERSALT equations to 0.40 for the Kawasaki, Tanaka, and Mage equations.

DISCUSSION

These data suggest that methods based upon spot urine samples may be used to estimate population salt intake in urban and rural areas of North and South India. For

all equations, bias was evident such that compared with estimates based upon 24-hour urine samples, spot urine estimates were higher at lower levels of salt intake and lower at higher intake levels, aside from the Mage equation where the direction of the bias was reversed. Regardless, except for the Kawasaki and Toft equations, group level estimates based upon spot urine equations were reasonably comparable to the 24-hour urine collection. This reflects the potential for even flawed individual measurements to still provide group level data of value.

Measurement of salt intake in individuals is challenging due to the significant day-to-day variation in dietary intake and variability in factors that influence sodium absorption, metabolism and excretion [8]. Measurement of salt intake using spot urine samples might be anticipated to introduce additional errors because it measures sodium at just one point in time and requires the inclusion of other variables, rather than simply averaging sodium across a 24-hour period. The narrower 95% confidence intervals and smaller standard deviations obtained with most of the spot urine-based estimates [10, 21-23] are therefore somewhat counterintuitive since they infer that a more precise estimate of usual salt intake is obtained. This is likely explained by the other variables that are included in these equations such as weight, body mass index and sex that are strongly associated with salt intake and either invariable or much less variable [24, 25].

These analyses show that the Kawasaki equation greatly overestimates salt intake when compared to 24-hour urine collections. This may be because the Kawasaki

equation was originally developed to be used on second-morning voids and the current study used morning, but not necessarily second morning, voids. The Toft equation also over-estimated salt intake in this Indian cohort. The Toft equation was developed in a Danish population, which is ethnically dissimilar to an Indian population and may explain the poorer agreement observed with this equation. A previous meta-analysis showed that there is heterogeneity in the estimation of mean population salt intake based on the spot urine equation used, and also that salt intake estimates derived from the Kawasaki equation significantly overestimated salt intake compared with 24-hour urine collections and other equations [9]. That overview showed a similar pattern of differences between mean population salt intake estimated using 24-hour urine samples and mean population salt intake estimated using equations to that observed here. In particular, the Kawasaki equation resulted in a large over-estimation. Likewise, at the individual level, there was strong evidence of bias in the estimates across different levels of salt intake and significant variation in the agreement between salt intake estimated by spot urine equations and 24-hour urine samples as shown by the wide limits of agreement. The proportional bias issue is hard to correct for because the bias varies across the spectrum of intake and highlights the need for a new equation that better predicts salt intake across a broad range of intakes.

Measuring and monitoring population salt consumption is an important aspect of salt reduction strategies [26]. Determining population salt consumption levels enables an objective decision to be made about whether or not salt reduction is a priority and also provides a baseline with which to measure the effectiveness of salt reduction

strategies. The World Health Organization's STEPwise approach to Surveillance (STEPS) protocol, which is used for health status monitoring globally, includes spot urine collection for estimation of salt intake [27]. However, in this protocol, there are no instructions regarding the timing of the urine samples or which spot urine equation should be used to estimate salt intake. Our data suggest that in an Indian population use of either the Tanaka, Mage or INTERSALT equations with a morning spot urine sample will provide a reasonable estimate of mean population salt intake. Further clarification on the optimal collection time and use of spot urine samples is required to inform population estimation of salt intake using methods based on spot urines. In addition, it is not clear whether spot urine samples can be used to detect changes in salt intake over time. In an Australian population, we showed that methods based upon spot urine samples can be used to detect changes in salt intake if the same individuals are sampled at each time point [21]. Similarly, in a Vietnamese cohort a similar change in salt intake, in response to a population-based intervention, was observed with methods based on spot urine samples and 24-hour urine collections [28]. This issue requires further investigation.

These analyses provide robust data about the use of spot urine samples for measurement of population salt intake in India. Urban and rural areas in Delhi and Haryana, and Andhra Pradesh, were sampled to provide data that are representative of North and South Indian populations. However, it is unclear whether these results can be generalized to populations from other ethnic and geographical areas. It should be noted that the spot urine samples were collected as part of the 24-hour urine

collections and therefore may over-estimate the agreement. Furthermore, all the urine samples were collected in the morning so it was not possible to determine the effect of urine collection timing. In this study, a large number (n=438; 31%) of 24-hour urine samples were assessed as incomplete on the basis of urine volume and creatinine assays. Para-amino benzoic acid was not used to assess the completeness of the 24-hour urine samples and it is possible that this would have resulted in further exclusions of incomplete samples.

In this large population-based sample of individuals from urban and rural areas in North and South India, some methods based upon spot urine samples provided a broadly comparable estimate of population salt intake to 24-hour urine collections. Methods based on spot urine samples present a viable alternative to measuring population salt intake, but whether changes in population salt intake over time can be detected by spot urine based methods requires future investigation. In addition, development of one equation, which is less biased and could be universally applied to estimate salt intake from spot urine samples would significantly further population monitoring of salt consumption.

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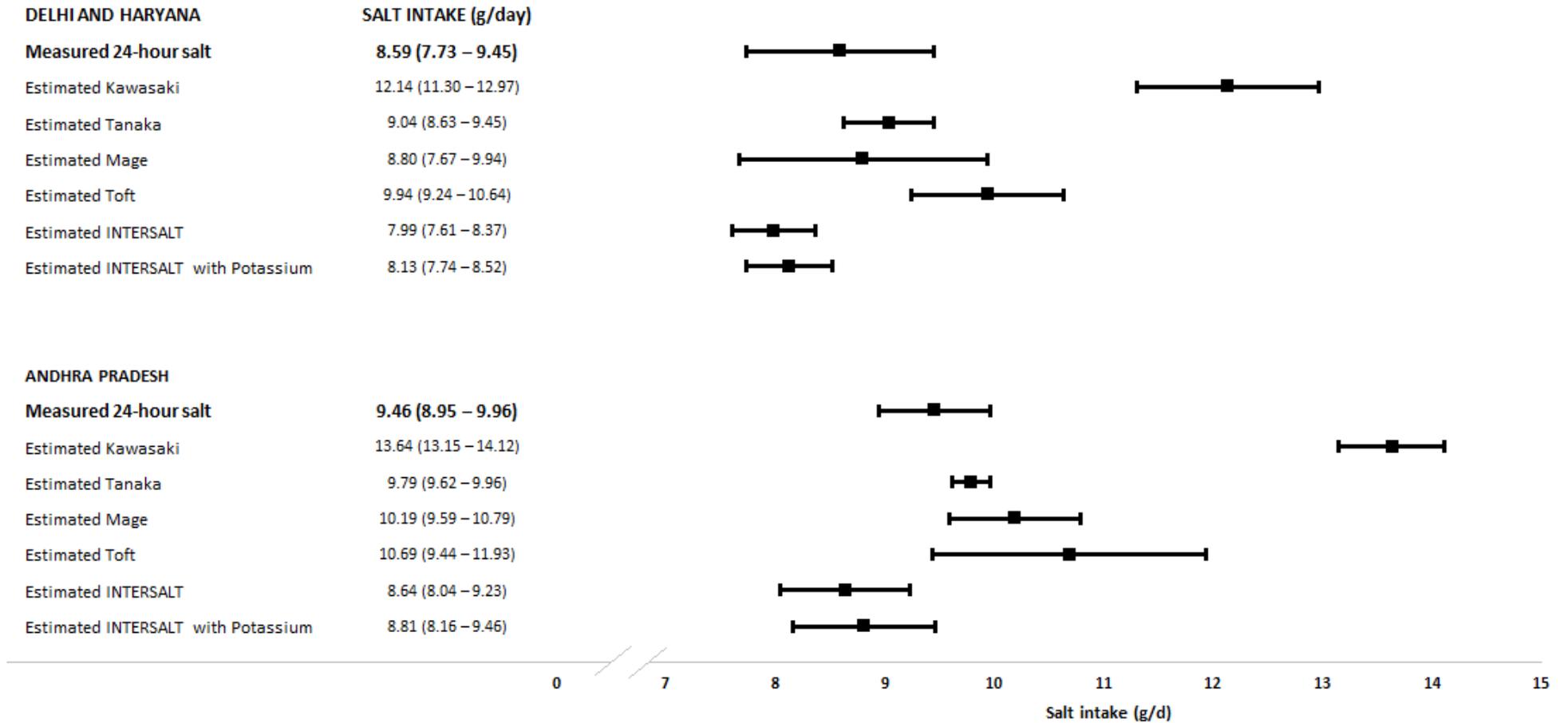
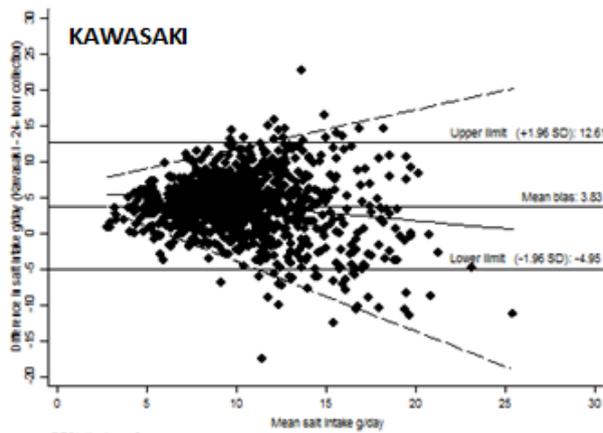
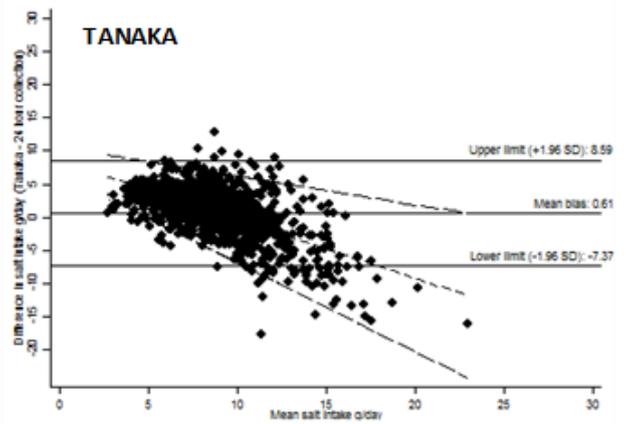


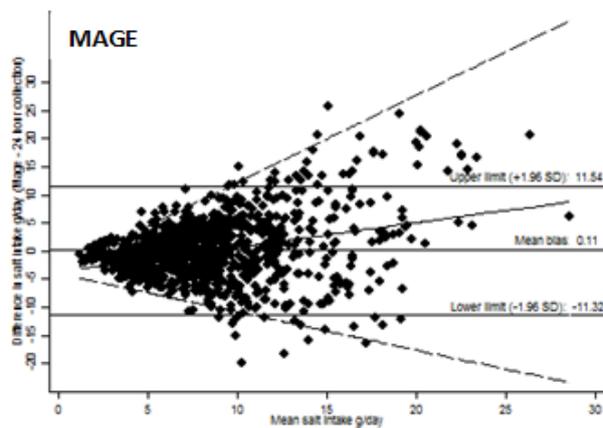
FIGURE 1: Overall weighted mean (95% confidence interval) salt intake estimated from 24-hour urine samples and spot urine sample for Delhi and Haryana, and Andhra Pradesh



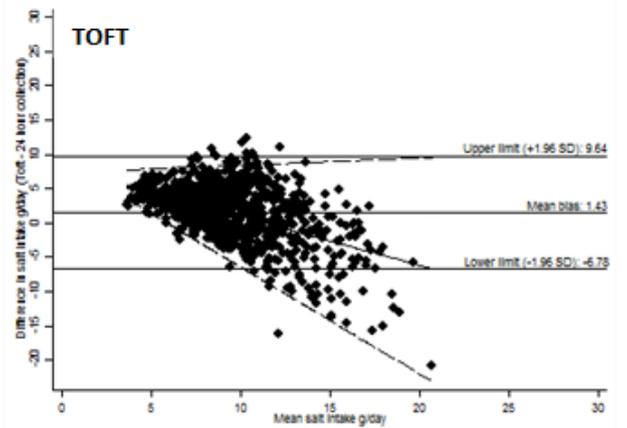
--- 95% limits of agreement
 Upper limit: $y = 6.35 + 0.54 \times \text{mean salt intake}$
 Lower limit: $y = 5.85 + -0.97 \times \text{mean salt intake}$



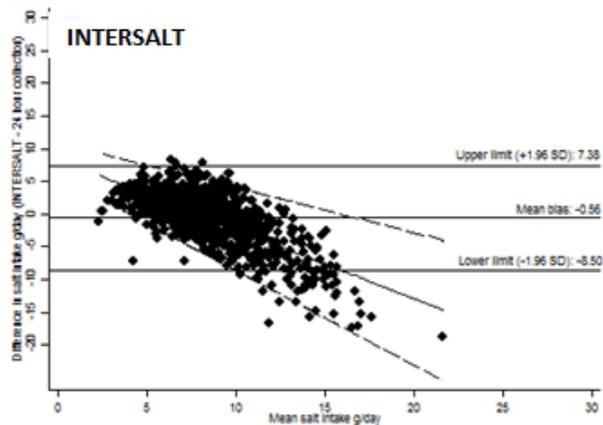
--- 95% limits of agreement
 Upper limit: $y = 10.56 + -0.43 \times \text{mean salt intake}$
 Lower limit: $y = 6.50 + -1.34 \times \text{mean salt intake}$



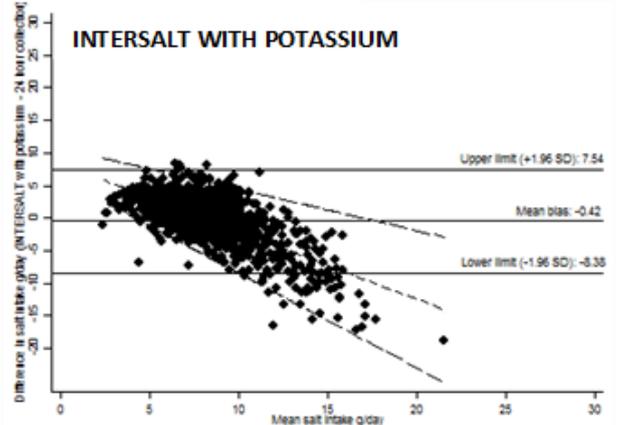
--- 95% limits of agreement
 Upper limit: $y = -3.31 + 1.55 \times \text{mean salt intake}$
 Lower limit: $y = -4.03 + -0.68 \times \text{mean salt intake}$



--- 95% limits of agreement
 Upper limit: $y = 7.33 + 0.11 \times \text{mean salt intake}$
 Lower limit: $y = 9.06 + -1.55 \times \text{mean salt intake}$



--- 95% limits of agreement
 Upper limit: $y = 10.83 + -0.68 \times \text{mean salt intake}$
 Lower limit: $y = 5.77 + -1.44 \times \text{mean salt intake}$



--- 95% limits of agreement
 Upper limit: $y = 10.71 + -0.63 \times \text{mean salt intake}$
 Lower limit: $y = 6.02 + -1.45 \times \text{mean salt intake}$

FIGURE 2: Bland-Altman plots of salt intake estimated based on 24-hour urine collection and spot urine samples

TABLE 1: Participant characteristics

	Delhi and Haryana	Andhra Pradesh
Age years (95% CI)	47 (46 – 49)	46 (44 – 47)
Male n (%)	195 (47)	280 (52)
Weight kg (95% CI)	62.9 (61.5 – 64.2)	61.7 (60.5 – 62.8)
Body mass index kg/m ² (95% CI) ¹	24.9 (24.4 – 25.4)	24.6 (24.2 – 25.1)
Body mass index classification ¹		
Healthy weight ($\leq 24.9\text{kg/m}^2$)	231 (56)	313 (58)
Overweight (25-29.9kg/m ²)	116 (28)	163 (30)
Obese ($\geq 30\text{kg/m}^2$)	67 (16)	64 (12)
Blood pressure n (%)		
Systolic blood pressure $\geq 140\text{mmHg}$ or diastolic blood pressure $\geq 90\text{mmHg}$	128 (30)	157 (29)
Systolic blood pressure $< 140\text{mmHg}$ and diastolic blood pressure $< 90\text{mmHg}$	289 (70)	383 (71)

¹n=414