



Original Article

Estimating mean change in population salt intake using spot urine samples

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Accepted 27 July 2016

Abstract

Background: Spot urine samples are easier to collect than 24-h urine samples and have been used with estimating equations to derive the mean daily salt intake of a population. Whether equations using data from spot urine samples can also be used to estimate change in mean daily population salt intake over time is unknown. We compared estimates of change in mean daily population salt intake based upon 24-h urine collections with estimates derived using equations based on spot urine samples.

Methods: Paired and unpaired 24-h urine samples and spot urine samples were collected from individuals in two Australian populations, in 2011 and 2014. Estimates of change in daily mean population salt intake between 2011 and 2014 were obtained directly from the 24-h urine samples and by applying established estimating equations (Kawasaki, Tanaka, Mage, Toft, INTERSALT) to the data from spot urine samples. Differences between 2011 and 2014 were calculated using mixed models.

Results: A total of 1000 participants provided a 24-h urine sample and a spot urine sample in 2011, and 1012 did so in 2014 (paired samples $n = 870$; unpaired samples $n = 1142$). The participants were community-dwelling individuals living in the State of Victoria or the town of Lithgow in the State of New South Wales, Australia, with a mean age of 55 years in 2011. The mean (95% confidence interval) difference in population salt intake between 2011 and 2014 determined from the 24-h urine samples was -0.48 g/day (-0.74 to -0.21 ; $P < 0.001$). The corresponding result estimated from the spot urine samples was -0.24 g/day (-0.42 to -0.06 ; $P = 0.01$) using the Tanaka equation, -0.42 g/day (-0.70 to -0.13 ; $p = 0.004$) using the Kawasaki equation, -0.51 g/day (-1.00 to -0.01 ; $P = 0.046$) using the Mage equation, -0.26 g/day (-0.42 to -0.10 ; $P = 0.001$) using the Toft equation, -0.20 g/day (-0.32 to -0.09 ; $P = 0.001$) using the INTERSALT equation and -0.27 g/day (-0.39 to -0.15 ; $P < 0.001$) using the INTERSALT equation with potassium. There was no evidence

that the changes detected by the 24-h collections and estimating equations were different (all $P > 0.058$). Separate analysis of the unpaired and paired data showed that detection of change by the estimating equations was observed only in the paired data.

Conclusions: All the estimating equations based upon spot urine samples identified a similar change in daily salt intake to that detected by the 24-h urine samples. Methods based upon spot urine samples may provide an approach to measuring change in mean population salt intake, although further investigation in larger and more diverse population groups is required.

Key words: Salt intake, sodium, spot urine collection, 24-h urine collection

Key Messages

- The use of spot urine samples to measure change in population salt intake may be a more feasible alternative to repeat 24-h urine collections.
- All of the established estimating equations based upon spot urine samples identified a similar change in salt intake to that determined from the 24-h urine collections.
- The utility of spot urine samples may be limited to matched samples, and additional analyses in diverse populations using larger datasets are required to better quantify the validity of methods based upon spot urine samples.

Introduction

High blood pressure is a leading cause of global disease burden,¹ and excess salt consumption is a well-established cause of raised blood pressure. It was recently estimated that salt consumption above the World Health Organization's recommended maximum of 5 g/day causes 1.65 million deaths each year, comprising 9.5% of all cardiovascular disease deaths worldwide.² Salt reduction has also been identified as a global health priority because it is projected to be one of the most cost-effective strategies for reducing blood pressure and vascular disease.³

All member states of the World Health Organization have agreed to a global target to reduce mean population salt intake by 30% by 2025.⁴ Integral to achieving this goal is being able to measure salt intake accurately enough to track changes in population salt intake over time. The collection of 24-h urine samples is currently the accepted best method for determining daily salt intake in an individual or population, especially if replicated using multiple samples to account for within-individual variability.⁵ It is also the currently accepted best method for detecting change in population salt over time. However, although 24-h urine samples may be a good method for determining salt excretion in clinical settings with highly trained staff, it can be a suboptimal method for the monitoring of general populations in community surveys. The method is burdensome for subjects, which can limit participation and affect

the representativeness of participants in population surveys.⁶ Incomplete collection of 24-h urine samples is also very common and results in an underestimation of true salt intake.⁷

Equations that estimate 24-h salt intake from a spot urine sample may provide a reasonable estimate of mean population consumption levels, and use of them may increase participation rates in surveys.^{8–11} However, compared with measures based upon 24-h urine samples, the equations tend to overestimate salt intake at lower levels of consumption and to underestimate intake at higher levels of consumption.¹¹ This proportional bias would be expected to result in an underestimate of the change in salt intake over time, when estimating equations are used instead of 24-h urine collections. It also raises questions about the validity of cohort studies that use these estimating methods to draw inferences about the association of salt intake with disease risk.¹² The greater within-person variability in spot urine samples compared with 24-h urine samples may impact upon the power to detect changes in mean population salt intake over time. The aim of this study was to determine the estimated change in mean population salt intake in two selected Australian populations using standard measures based upon 24-h urine samples and to compare the findings to estimates made using measures based upon spot urine samples.

Methods

This work used data collected as part of an Australian National Health and Medical Research Council-supported project designed to measure the effectiveness of local, regional and national interventions to reduce salt consumption in Australia. The data are derived from surveys conducted among individuals in the State of Victoria and the town of Lithgow in the State of New South Wales. In Lithgow, a community-based salt reduction programme was implemented between 2011 and 2014,¹³ which reduced population salt consumption. The Victorian study was not accompanied by a local salt reduction programme and no change in salt intake was observed between 2011 and 2014.¹⁴ The project was done between 2010 and 2015 and was reviewed and approved by the ethics committees of the University of Sydney, Alfred Hospital and Deakin University. All survey participants provided written informed consent and the study was registered at [<http://clinicaltrials.gov>] (NCT02105727).

Participants

Participants were community-dwelling individuals aged over 18 years. There were no exclusions based on use of medications or any other aspect of demography or personal or medical history. The sampling methodology has been previously published.^{13,14} Briefly, the participants enrolled at baseline in 2011 were a mix of individuals who were randomly sampled and those who volunteered to participate. For the follow-up survey in 2014, individuals who were involved in the baseline survey were invited and additional participants were recruited by random sampling and community advertisements. Therefore we have individuals who participated in both 2011 and 2014 (paired data) and a sample that participated in either 2011 or 2014 (unpaired data).

Urine sample collection

A single 24-h urine sample and a single spot urine sample were collected at each participant contact. The 24-h urine collection was obtained by discarding the first voided urine upon waking on the day of collection and then collecting all voided urine up to and including the first void the following morning. The times at the beginning and the end of urine collection were recorded and the time-adjusted 24-h salt excretion was calculated. For each individual, the 24-h sodium excretion value (mmol/day) was calculated as the concentration of sodium in the urine (mmol/l) multiplied by the urinary volume (l/day) multiplied by the time adjustment factor. 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 same process was used for urine samples collected in both Victoria and Lithgow. Suspected incomplete 24-h urine collections (i.e. urinary creatinine < 4.0 mmol/day for women, or < 6.0 mmol/day for men or a 24-h urine collection of < 500 ml for either gender) and suspected over-collections (i.e. urinary creatinine or collection volumes > 3 standard deviations above the population mean) were excluded from the analysis.

Participants performed 24-h urine collections and collected a spot collection in a separate container within this period. Participants from Victoria were randomly allocated to collect their spot urine either after their evening meal or just before bedtime. Participants in Lithgow performed their spot collection at the second morning void. The spot urine sodium concentration was measured separately and the volume of the spot urine samples was recorded. The volume and concentration of the spot urine samples were added to the remainder of the 24-h data to derive the full 24-h excretion.

Estimating equations for spot urine samples

Daily salt excretion was estimated from the spot urine specimens by applying a series of established predictive equations that use the concentration of sodium in the spot urine along with a range of other variables. The equations tested were: Tanaka,¹⁵ Kawasaki,¹⁶ Toft,¹⁷ Mage,^{18,19} INTERSALT without potassium and INTERSALT with potassium⁹ (see [Supplementary Table 1](#), available as [Supplementary data](#) at IJE online). All of these equations also incorporate urinary creatinine concentration, age, weight and height [or body mass index (BMI)]. In addition, most provide separate equations for each sex (Kawasaki, Mage, Toft, INTERSALT) and two include modifications by ethnicity (INTERSALT and Mage).

Statistical methods

For each sodium measurement method (24-h urines or estimating equations based upon spot urine samples), mixed effect modelling was used to make estimates of the difference between mean population salt intake levels in 2011 and 2014. No adjustments were made for age, sex, weight or BMI. This method allows for paired and unpaired data to be analysed jointly. Participant characteristics were compared between 2011 and 2014 using the same method for the continuous variables and Pearson's chi square test for the gender distribution. Using paired *t* tests (paired data only), the change estimate provided by the equations

Table 1. Participant characteristics

	<i>n</i>	2011	<i>n</i>	2014	<i>P</i> -value ^a
Mean age (95% confidence interval), years					
All	1000	55 (55 to 56)	1012	58 (58 to 59)	< 0.001
Paired	435	58 (57 to 60)	435	61 (60 to 63)	< 0.001
Unpaired	565	55 (54 to 56)	577	56 (55 to 58)	0.12
Male <i>n</i> (%)					
All	1000	452 (45)	1012	445 (44)	–
Paired	435	201 (46)	435	201 (46)	–
Unpaired	565	251 (44)	577	244 (42)	0.47 ^b
Mean weight (95% confidence interval), kg					
All	1000	79.3 (78.4 to 80.2)	1012	79.4 (78.4 to 80.3)	0.75
Paired	435	78.0 (76.4 to 79.5)	435	77.9 (76.3 to 79.5)	0.87
Unpaired	565	79.2 (77.8 to 80.7)	577	80.5 (78.9 to 82.2)	0.24
Mean BMI (95% confidence interval), kg/m²					
All	1000	28.1 (27.8 to 28.3)	1012	28.2 (27.8 to 28.5)	0.25
Paired	435	27.4 (26.9 to 27.9)	435	27.4 (26.9 to 27.9)	0.86
Unpaired	565	28.0 (27.6 to 28.5)	577	28.8 (28.2 to 29.3)	0.03

^aMixed effect modelling for 2014 vs 2011.^bPearson chi-square test for 2014 vs 2011.

based upon spot urine samples were compared with the change estimated from the 24-h urine samples. Using the paired data only, the Bland-Altman method²⁰ was used to plot the difference between the change estimates provided by the equations based upon spot urine samples and the 24-h urine samples against the mean change estimates derived from the two methods. A regression line was fitted to these data to determine the degree of bias in spot urine-based results at any level of change. Using the paired data only, a one-way analysis of variance was used to determine if there was any difference in the change estimate derived from the estimating equations based on the timing of the spot urine collection (second morning, evening, bedtime). Statistical analyses were conducted using SPSS for Windows (Version 23, SPSS Inc., Chicago, IL). A *P*-value of < 0.05 was deemed unlikely to have arisen by chance.

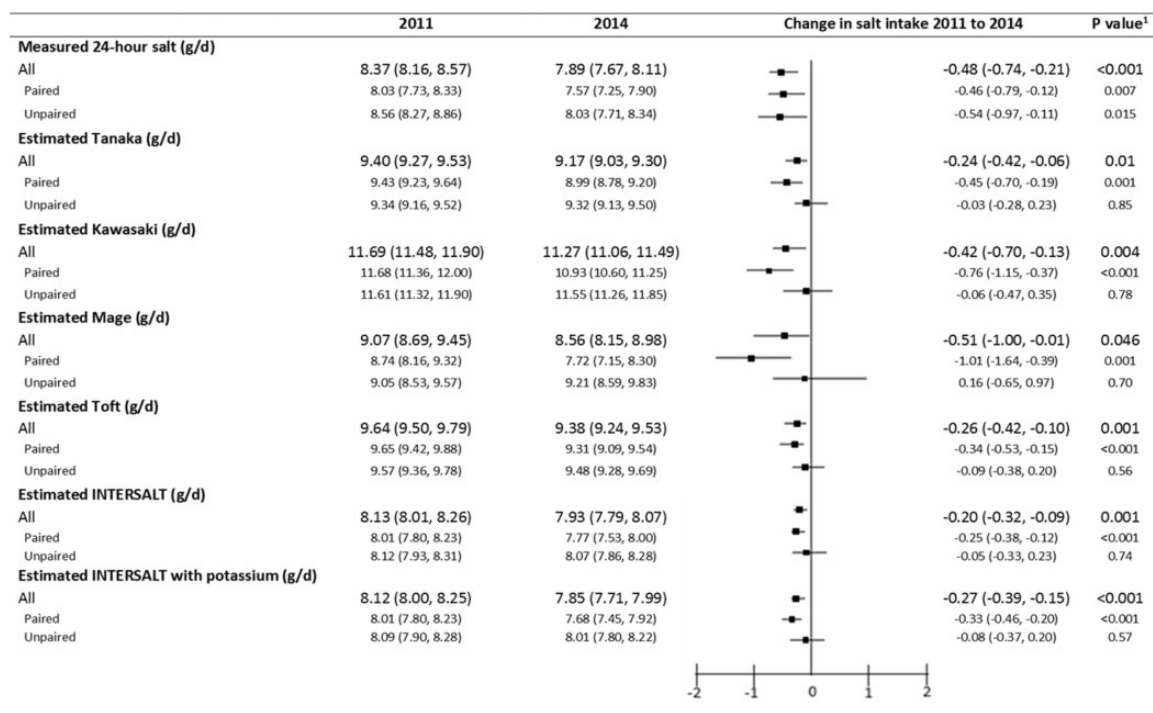
Results

There were 1000 eligible participants in 2011 and 1012 in 2014. This comprised 435 individuals from whom samples were collected in both 2011 and 2014 (Lithgow *n* = 135; Victoria *n* = 300), 565 individuals who provided samples in 2011 alone (Lithgow *n* = 280; Victoria *n* = 285) and 577 individuals who provided samples in 2014 alone (Lithgow *n* = 446; Victoria *n* = 131). These numbers exclude 36 individuals for whom the 24-h urine specimens were suspected to be incomplete, 34 without a spot urine sample, 2 because height was not recorded and 3 because weight was not recorded.

Participants were on average approximately 3 years older in 2014 compared with 2011 for both the Lithgow and Victoria population samples (Table 1). In Lithgow, this difference was driven by the ageing of the participants studied in both 2011 and 2014 (the paired subset) but was apparent for both the paired and unpaired subset in the Victoria sample (Supplementary Table 2, available as Supplementary data at IJE online). The proportions of male gender, mean weight and mean BMI were not different between 2011 and 2014 for the overall sample, the Lithgow sample alone or the Victoria sample alone. Participation rates in the Lithgow and Victoria surveys for the 24-h urine collections were 16% and 38%, respectively.^{14,21}

Estimated difference in mean population salt consumption

The overall mean difference in population salt intake between 2011 and 2014 determined from the 24-h urine samples was –0.48 g/day (–0.74 to –0.21; *P* < 0.001) (Figure 1) primarily as a consequence of a reduction observed in the Lithgow sample. The corresponding mean differences estimated from the spot urine samples were –0.24 g/day (–0.42 to –0.06; *P* = 0.01) using the Tanaka equation, –0.42 g/day (–0.70 to –0.13; *P* = 0.004) using the Kawasaki equation, –0.51 g/day (–1.00 to –0.01; *P* = 0.046) using the Mage equation, –0.26 g/day (–0.42 to –0.10; *P* = 0.001) using the Toft equation, –0.20 g/day (–0.32 to –0.09; *P* = 0.001) using the INTERSALT equation and –0.27 g/day (–0.39 to –0.15; *P* < 0.001) using the INTERSALT equation with potassium.



Data presented as mean (95% confidence interval); ¹Mixed effect modelling for 2014 vs. 2011; Whole cohort: 2011 n=1000, 2014 n=1012; Paired analysis: 2011 & 2014 n=435; Unpaired analysis: 2011 n=565, 2014 n=577

Figure 1. Change in mean daily population salt intake (g/d) from 2011 to 2014 measured using 24-hour urine collections and estimated from spot urine samples.

Based on 24-h urine samples, there were similar reductions in estimated daily mean salt intake for both the paired and the unpaired subsets of individuals (Figure 1). For the estimating equations, however, reductions were observed only for the estimates based upon the paired data ($P < 0.01$). For the paired data, the difference in salt intake estimated by the equations based upon spot urine samples were comparable to the difference estimate provided by the 24-h urine samples for all comparisons (all $P > 0.058$).

Bland-Altman plots showed that the difference in the change estimates detected by the two methods were not consistent across different levels of change. In every case, the size of the bias was directly proportional to the size of the change, although the direction of the bias was not consistent (Figure 2). Although the absolute magnitude of the bias was fairly small for small differences, it resulted in large discrepancies between the estimates when the mean change was large. Using the paired data only, the change estimate according to the time of the spot urine collection was investigated. There was no difference between the change estimates based on the timing of the spot urine collection (second morning, evening or bedtime) ($P > 0.32$ for all comparisons).

When the data were analysed separately by geographical location, the spot urine samples did not consistently detect the change in salt intake shown by the 24-h urine collections. For the analyses done separately on the Lithgow data alone, only the INTERSALT equations identified the reduction detected by the 24-h urine measurements (Supplementary Table 3, available as Supplementary data at IJE online). For both INTERSALT equations, the magnitude of the reduction measured by the 24-h urine samples was markedly underestimated. For the analyses of the Victoria data alone, all the equations identified the reduction detected by the 24-h urine measurements (Supplementary Table 4, available as Supplementary data at IJE online). As for the primary analyses that included both the unpaired and paired data, it was the application of the equations to the paired data that most consistently identified the reduction measured by the 24-h samples.

Discussion

These data suggest that spot urine samples may provide an alternative method for measuring changes in mean population salt intake over time. Our most robust test of this

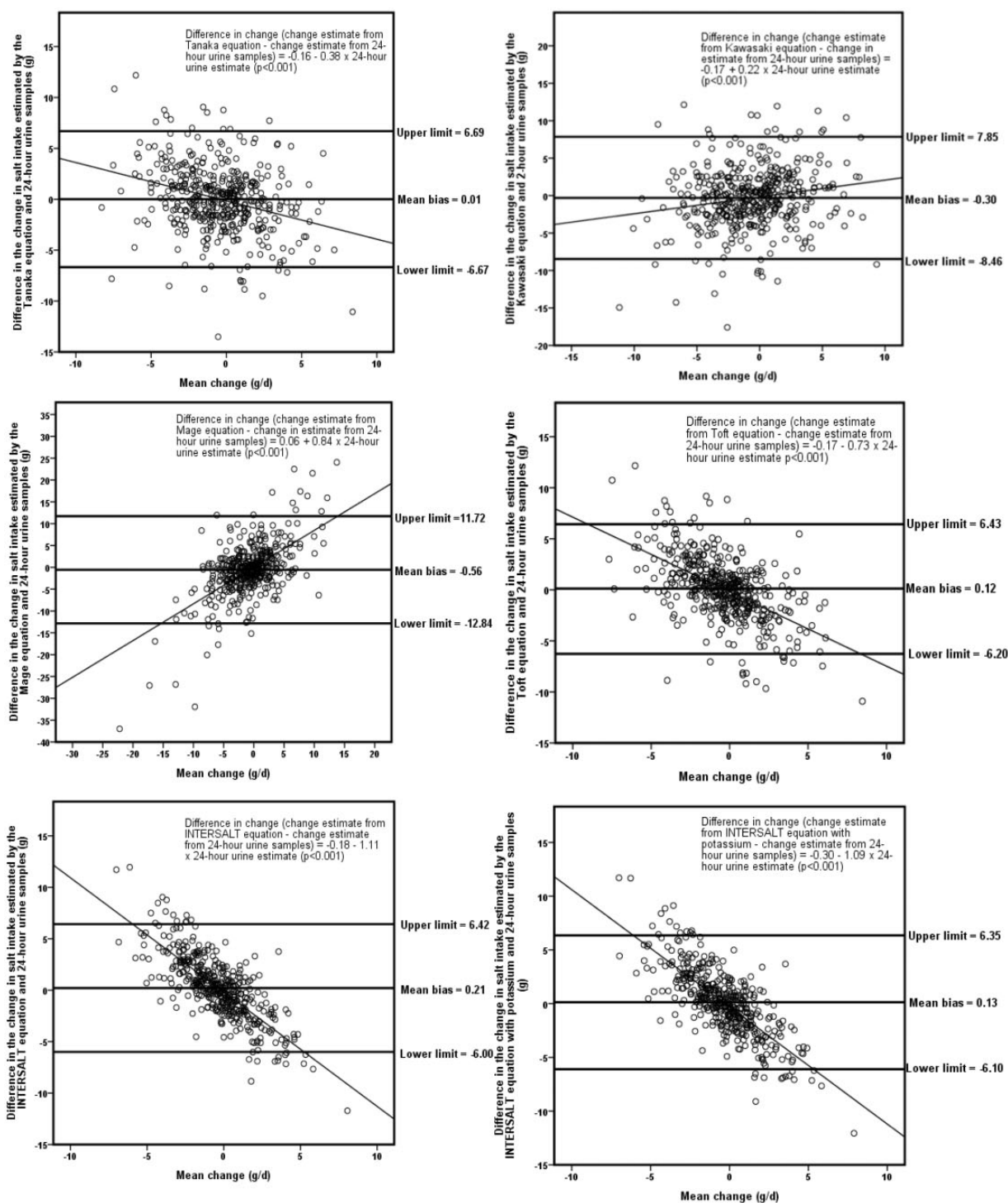


Figure 2. The difference between the change in mean population salt intake estimated from 24-hour and spot urine samples plotted against the mean of the change detected by both methods. Includes the paired data only.

hypothesis is that, based upon the large combined dataset and in these analyses, every estimation method based upon spot urine samples identified the difference in salt excretion documented by the 24-h urine samples. For all the predictive equations except the Mage equation, the point estimate of effect was numerically less than

that recorded for the 24-h urine samples, although the analysis of the paired data identified no differences in the change estimates derived from the estimating equations compared with those derived from the 24-h urine samples although the power of the analyses was limited.

These results add to the growing body of literature suggesting that spot urine samples may be a viable alternative to 24-h urine collections for population assessments of salt intake, although the findings derive from a single highly selected Australian population. Our previously conducted meta-analysis showed that estimates based upon spot urine samples can be used to determine whether mean population salt intake is above or below the 5 g/day target recommended by the World Health Organization, so that objective decisions about the requirement for salt reduction strategies can be made.¹¹ Our new analysis, using spot urine samples collected at serial time points, now provides the first evaluation of how spot urine samples might be used to monitor changes in population salt intake over time.

In our analyses, the absolute estimates of 24-h salt excretion based upon spot urine samples sometimes varied considerably from the levels based upon 24-h collections. For example, the Kawasaki equation estimated the mean 24-h salt excretion to be 11.7 g in 2011 and 11.3 g in 2014, compared with corresponding excretion measures based upon 24-h urine samples of 8.4 g and 7.9 g. Of note, however, whereas the absolute estimates derived using each method were quite disparate (about 11 g vs about 8 g), the within-method differences in mean daily intake over time were very similar at about -0.48 g by the 24-h urine samples and about -0.42 g using the Kawasaki equation. So, although substantial bias may be present at each measurement time point for the spot urine-based methods, this may not reduce the capacity of these equations to detect a change in salt intake over time if population samples are appropriately matched for key variables included in the predictive equations (i.e. weight, height, BMI, age, gender). This may also explain why, when the same individuals were studied (paired analyses), the point estimates of change provided by the spot urine samples were most comparable to the estimates provided by the 24-h urine samples.

Our findings align with what might be expected based upon the known characteristics of spot urine samples as a measurement method. The estimates based upon spot urine samples had larger P-values than those based upon 24-h urine samples. This is likely to be a consequence of the greater imprecision in measuring individual salt intake using spot urine samples compared with 24-h urine samples. Nonetheless, if it is mean population levels of intake (or change in intake) that are of interest, such as when estimating changes in population salt intake in response to a national salt reduction programme, then it should be possible to address greater imprecision at the individual level simply by using a larger sample size. Power calculations, that use the variability about 24-h urine values determined

from daily intake estimates made using simple arithmetic calculations based upon spot urine sample concentrations and urine volumes, suggest that about five times as many spot urines as 24-h urine samples would be required to achieve comparable power to detect a given reduction in salt intake ([Supplementary Table 5](#), available as [Supplementary data](#) at IJE online). However, this figure is unlikely to be correct because the daily salt intake estimates for individuals derived from the equations actually have less variability than the estimates derived from 24-h urine samples. The lower variability is likely because the outputs from the estimating equations are influenced by variables such as age, sex and BMI which are fairly constant. At the same time, because the equations include these variables that will not respond to change in daily salt intake, the equations may be less sensitive tools for detecting change in salt consumption over time. How these two aspects of equation performance will trade off is uncertain and will only be established by larger studies.

For salt intake estimation methods based upon spot urine samples, the situation is complicated by a further issue of bias identified by our previous meta-analysis of cross-sectional data.¹¹ Compared with 24-h urine samples, estimates based upon spot urine samples collected at the same time point systematically underestimate salt intake at high levels and systematically overestimate salt intake at low levels. The magnitude of this effect is such that a true reduction in salt excretion measured by 24-h urine samples of 10% (from 10 g/day) would be underestimated by about one-fifth and observed as an 8% reduction if measured using spot urine samples. An investigation of the paired data in the current analysis showed that bias was present when spot urine samples were used to measure the change in salt intake over time, although the direction of the bias was not consistent. The biases in spot urine estimates of 24-h salt excretion suggested by the current analysis, and the previous work done in the field,¹¹ highlight the need for further research and the investigation of possible new and better equations that can provide reliable estimates across a broad range of salt intake levels.

Currently there are six established equations which can be used to estimate 24-h salt intake from a spot urine sample and there is no clarity about which equation to use. This is an important issue because the World Health Organization has added spot urine samples to the STEPwise approach to Surveillance (STEPS) protocol for measurement of salt intake. Our previous meta-analysis showed heterogeneity in estimates of salt intake based on the spot urine equation used,¹¹ and analyses of the paired data in the current study suggested comparable difference estimates across all the estimating equations and 24-h urine samples, but statistical power to detect variation

across the equations was limited. It might have been anticipated that the INTERSALT and Toft equations would perform best on these data which derive from Australian populations broadly comparable in characteristics to the North American and Danish populations from which the INTERSALT and Toft equations were derived.¹⁷ This certainly seems to be true for the estimation of absolute levels of intake, but these equations did not appear to perform better in estimating change in mean intake over time.

In the analysis which included both the New South Wales and Victorian data, a reduction in salt intake of 0.48 g/day was shown between 2011 and 2014. This should not be misinterpreted to suggest that there has been a population reduction in salt intake in Australia during this time. This result is largely driven by the data from one town in New South Wales where a targeted salt reduction intervention reduced population salt intake, and no comparable effect was observed in Victoria.¹⁴

To maximize the sample size available for these analyses, both unpaired and paired data were used for the primary analyses. However in practice, serial surveys of population salt consumption are likely to include different individuals drawn from the same population on each occasion (unpaired data). The estimates of change based upon unpaired spot urine samples reported here were unconvincing although the sample size was insufficiently large to robustly test the unpaired data alone. Additional data from unpaired analyses are required to clarify if the apparent difference between the findings for the paired and unpaired analyses are real or the consequence of chance. The completeness of the 24-h urine collections was not verified using para-aminobenzoic acid.²² The relatively small sample size precluded us from completing subgroup analyses to explore the consistency of the findings across groups defined by age, sex and other characteristics. Spot urine collections occurred at different times of the day (morning, evening, bedtime) with about two-thirds of spot samples collected in the evening. Cross-sectional data from a previous study⁸ showed that spot urine-based estimates of 24-h excretion derived from afternoon and evening spot samples performed better than morning or overnight spot samples. This may explain some differences in the findings for the Victoria compared with the Lithgow data, even though analyses conducted using the paired data showed no difference in the change estimate according to the time of the spot urine collection.

Conclusion

All the estimating equations based upon spot urine samples identified the reduction in daily salt intake detected by the 24-h urine samples, and these data suggest that methods

based upon spot urine samples may provide an approach to measuring change in mean population salt intake over time. However, the findings appeared to be restricted to analyses based upon repeat assessment of the same individuals, and additional analyses in diverse populations using larger datasets are required to better quantify how approaches based upon spot urine samples perform. It also seems likely that with a large dataset it will be possible to develop new, less biased equations that are specifically designed to detect change over time using spot urine samples.

Supplementary Data

Supplementary data are available at IJE online.

Funding

This work was supported by a National Health and Medical Research Council of Australia (NHMRC) Program Grant (APP1052555) and data collection was funded by an NHMRC Partnership Project Grant (APP571439). B.N. is supported by an NHMRC Principal Research Fellowship (APP1106947). J.W. is supported by a joint NHMRC and Heart Foundation Career Development Fellowship (1082924).

Conflict of interest: J.W. is the Director of the World Health Organization Collaborating Centre on Population Salt Reduction, with a remit to support countries to achieve the global targets of a 30% reduction in population salt intake by 2025. M.W. is a consultant to Amgen.

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