Methodological Issues in Cohort Studies That Relate Sodium Intake to Cardiovascular Disease Outcomes: A Science Advisory From the American Heart Association

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on behalf of the American Heart Association Council on Lifestyle and Metabolic Health

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AHA Science Advisory

Methodological Issues in Cohort Studies That Relate Sodium Intake to Cardiovascular Disease Outcomes

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Background—The results of cohort studies relating sodium (Na) intake to blood pressure–related cardiovascular disease (CVD) are inconsistent. To understand whether methodological issues account for the inconsistency, we reviewed the quality of these studies.

Methods and Results—We reviewed cohort studies that examined the association between Na and CVD. We then identified methodological issues with greatest potential to alter the direction of association (reverse causality, systematic error in Na assessment), some potential to alter the direction of association (residual confounding, inadequate follow-up), and the potential to yield false null results (random error in Na assessment, insufficient power). We included 26 studies with 31 independent analyses. Of these, 13 found direct associations between Na and CVD, 8 found inverse associations, 2 found J-shaped associations, and 8 found null associations only. On average there were 3 to 4 methodological issues per study. Issues with greater potential to alter the direction of association were present in all but 1 of the 26 studies (systematic error, 22; reverse causality, 16). Issues with lesser potential to alter the direction of association were present in 18 studies, whereas those with potential to yield false null results were present in 23.

Conclusions—Methodological issues may account for the inconsistent findings in currently available observational studies relating Na to CVD. Until well-designed cohort studies in the general population are available, it remains appropriate to base Na guidelines on the robust body of evidence linking Na with elevated blood pressure and the few existing general population trials of the effects of Na reduction on CVD. **(*Circulation*. 2014;129:1173-1186.)**

Key Words: AHA Scientific Statements ■ cardiovascular diseases ■ coronary diseases ■ diet ■ sodium ■ stroke

The relationship between sodium (Na) intake and blood pressure (BP) is well established, based on a diverse body of evidence including clinical trials.[1] Meta-analyses of randomized controlled trials in both adults and children have found that reducing Na can lead to important reductions in BP.[2–6] In addition, trials have consistently identified a clear dose-response relationship between Na intake and BP, with progressively lower levels of Na intake being associated with lower levels of BP.[7–9]

Trials that test the efficacy of reduced Na intake on clinical cardiovascular disease (CVD) outcomes in general populations have found that reduction of Na intake is associated with lower CVD, although these trials are few and underpowered.[10] Only 1 published trial, which substituted potassium for ≈50% of dietary Na, was specifically designed to address this question.[11] Six other trials, primarily designed to study the long-term relationship between Na intake and BP, have also

*The views expressed are those of the author and not necessarily those of the National Health Service (United Kingdom), the National Institute for Health Research (United Kingdom), or the Department of Health (United Kingdom).

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reported the effects of Na intake on CVD outcomes, which have subsequently been used in meta-analyses.[10,12] Although trials designed to address this gap would be ideal,[12,13] sample size requirements, cost, and the difficulty of sustaining a long-term contrast in Na intake between treatment groups make this challenging.[10,14] To the best of our knowledge, only 1 trial specifically designed to test the efficacy of Na reduction on CVD outcomes is under way, in China.[15]

A 2009 meta-analysis of cohort studies documented a significant direct relationship of Na intake with CVD outcomes[16]; a more recent meta-analysis that also included trial evidence concluded that lower Na intake is associated with a reduced risk of stroke and fatal coronary heart disease (CHD) in adults, although the quality of the evidence was deemed low.[2] Both found substantial heterogeneity in the results across studies, a component of which resulted from conflicting reports by authors who conducted separate analyses of the same data sets.[17–20] Hence, it is plausible that some of this heterogeneity results from differences in analytic strategy as well as study design. Since the 2009 meta-analysis, 13 additional studies have been published, with 6 showing either an inverse or J-shaped association between Na intake and CVD.

Letters, editorials, and scientific statements have offered methodological critiques of individual studies, but there has been no systematic assessment of the quality of the available studies.[14,21,22] The objective of the present report was to assess the quality of cohort studies examining the relationship between Na intake and subsequent CVD and to describe the potential contribution of methodological issues to the heterogeneity of results. To prepare this report, the American Heart Association assembled a group of investigators who were familiar with methodological challenges inherent in the design, conduct, and analysis of prospective studies that relate Na intake to CVD.

Methods

Because many of the methodological challenges pertain to measuring Na intake, we provide a brief overview of the techniques used to estimate Na intake in cohort studies.

Estimating Na Intake

The 2 main approaches are urine collections and dietary surveys. Na intake varies widely from day to day; consequently, although a single day's measurement can be useful in characterizing group intake, it is too imprecise to assess an individual's usual intake.[23] Relying on a single day of data can lead to random errors in Na assessment.

Averaging multiple 24-hour urinary Na collections provides the most accurate characterization of an individual's usual Na intake, because typically >90% of the Na consumed by healthy individuals is recovered in their urine.[23–25] Collection of even one 24-hour urine specimen, however, carries high participant burden. Therefore, to estimate Na intake, studies typically obtain a single collection of urine (24-hour, overnight, or "spot") rather than multiple collections. Besides the difficulty of assessing usual intake, 24-hour urine collections are often incomplete unless specific approaches are taken to avoid undercollection,[26,27] with 1 study reporting underestimation in 25% of its samples.[28] Both overnight and spot urine collections are easier for participants and less prone to be incomplete but have been shown to either underestimate (overnight collections)[29] or overestimate (spot urines)[30] 24-hour values. A recent systematic review found that their reliability varied widely.[31] Of particular concern, diurnal variation in Na excretion differs based on medication use[32] and clinical conditions[33,34] and can adversely impact the validity of these shorter collections as estimates of individual 24-hour Na excretion.

Methods for dietary assessment of Na intake include use of food records, 24-hour recalls, and food frequency questionnaires (FFQs). The ability of food records and 24-hour recalls to capture usual intake depends on how many days are assessed. FFQs aim to capture usual intake by asking about eating habits over months or years, but their accuracy is limited by the number and relevance of food items included, the lack of specific product information, and a high potential for recall bias.[35] In all diet surveys, error in estimating Na intake can arise from (1) inaccurate reporting by participants of the types and quantity of food that they have consumed (Na is highly correlated with calorie intake)[36]; (2) lack of inclusion of salt added at the table, in condiments, and in some instances during cooking[37]; and (3) reliance on incomplete and infrequently updated food composition tables to determine the Na content of food.[36] Underreporting of energy intake is particularly problematic in FFQs but is a problem in all dietary surveys.[38] Importantly, underreporting is often influenced by key study variables; for example, overweight and obese study participants commonly underestimate their food intake relative to their leaner counterparts.[39–42]

Description and Rationale of Methodological Issues

We identified 3 categories (domains) of methodological issues that apply to observational studies of Na and CVD: (1) Those with the greatest potential to alter the direction of association (in either direction); (2) those with some potential to alter the direction of association but of a lesser magnitude; and (3) those with the potential to lead to a false null result. Table 1 lists the 3 domains and the criteria we applied.

Domain 1: Errors With the Greatest Potential to Alter the Direction of Association

Systematic Error in Na Assessment

Systematic error arises when the measured overall mean Na differs from the true overall mean Na. If the systematic error differs by exposure or disease status, it can have unpredictable effects on estimation of the relationship between Na intake and CVD.[35] As noted above, all types of Na measurement are vulnerable to systematic error in estimating Na intake, although to different extents. Furthermore, the error in both dietary studies and partial urine collections has been shown to differ by clinical characteristics.

We classified studies into 2 groups based on the potential for systematic error inherent in their method of assessment. The first group, lower risk of systematic error, is limited to 24-hour urine collections not collected as part of routine clinical practice that report quality assurance or exclude incomplete collections. The second group, higher risk of systematic error, includes other 24-hour urine collections, all dietary assessment methods, and spot and overnight urine collections. Furthermore, regardless of the type of data

Table 1. Domain Criteria

Domain 1: Errors with the greatest potential to alter the direction of association in either direction

Potential for systematic error in Na assessment:

- High:
  - — Participants instructed to reduce Na intake or modify diet prior to Na assessment
  - — Na intake measured through food frequency questionnaires, 24-h recalls, food records, spot or overnight urine collections, or 24-h urine collections without evidence of quality control measures
  - — Evidence of systematic error, identified by an implausible difference in mean calories (in diet studies) or urinary creatinine excretion (for urine studies) compared with weight (or BMI) across levels of Na intake.
- Lower:
  - — 24-h urine collections with reported quality control measures

Potential for reverse causality:

- High:
  - — Specifically recruited sick participants (pre-existing CVD, diabetes mellitus, CHF, or ESRD)
  - — Removing sick participants from analysis changes direction of association
- Intermediate:
  - — Sick populations not excluded from general population study
  - — Evidence that despite exclusions, participants with prior CVD were included
  - — Recruitment of populations with existing CVD risk factors (eg, hypertension)
  - — Specifically recruited sick populations but assessed both violations of proportional hazards and excluded early events in sensitivity analysis
- Low: Recruited from the general population and preexisting CVD excluded from analysis

Domain 2: Errors with some potential to alter the direction of association in either direction

Potential for residual confounding 1: Incomplete adjustment:

- Yes:
  - — ≥2 of the following major risk factors for CVD: age, sex, race, SES, cholesterol, BMI (or weight), smoking, diabetes mellitus, and (if an RCT) treatment assignment not included in final model
  - — Diet-based studies that do not control for calories in multivariate models
  - — Urine-based studies that do not control for weight, BMI, or creatinine excretion
- Unlikely: Either no apparent errors or minor errors not included above

Potential for residual confounding 2: Study imbalance

- Yes:
  - — Age difference across Na intake groups is >5 y
  - — Sex or race distribution across Na intake groups differs by >20%
- Unlikely:
  - — Meets criteria above, but when stratified analyses are conducted on potential source of residual confounding, results do not differ
  - — Does not meet criteria above
- Cannot assess: No information provided on age, race, or sex by Na intake groups

(*continued*)

Table 1. Continued

Inadequate follow-up

- Yes: Low levels of follow-up (<80%) or follow-up of uncertain quality for CVD outcomes
- Unlikely: Good follow-up on ≥80% of participants

Domain 3: Errors with potential to lead to a false null result

Random error in Na assessment:

- High:
  - — Urine collection: <24 h, single 24-h urine measures
  - — Single 24-h dietary recalls or 1-d food records
- Intermediate:
  - — Urine collection: Two to four 24-h urine collections, or correction for regression dilution bias with second collection on a sample of participants
  - — Dietary reports: Multiple days of food records or dietary recalls; a single-day dietary report corrected for regression dilution bias with a second day in a sample of participants
- Low:
  - — Urine collection: More than four 24-h urine assessments on average
  - — Food frequency questionnaires

Insufficient power

- Yes: Study has <80% power to detect a 10% reduction in relative risk for every standard deviation drop in Na intake using a standard calculation (based on the maximum number of CVD events)
- Unlikely: Study has ≥80% power to detect a 10% reduction in relative risk for every standard deviation drop in Na intake

BMI indicates body mass index; CHF, congestive heart failure; CVD, cardiovascular disease; ESRD, end-stage renal disease; RCT, randomized controlled trial; SES, socioeconomic status; and Na, sodium.

collection, the potential for systematic error was determined to be high when participants were instructed to change their Na intake before Na assessment or if we observed systematic error in published results. We identified evidence of systematic error by an implausible difference in mean calories (in diet studies) or urinary creatinine excretion (for urine studies) compared with mean weight (or body mass index) across levels of Na intake.

Potential for Reverse Causality

Reverse causality in Na studies arises when sick individuals included in a study have reduced their Na intake either because of medical advice or an illness-related reduction in food consumption.[21] This may result in a J-shaped relationship leading to the misinterpretation that very low levels of Na intake have resulted in illness, when instead, it is likely that the illness is responsible for the low level of Na intake.[43] Reverse causality is more likely to be a problem in studies with a relatively high percentage of sick participants and when the study outcome is based on mortality rather than incident events.[43]

Although it is likely that some level of reverse causality exists in all cohort studies with diet as an exposure, we divided studies into 3 groups to reflect the likelihood that it biased the relationship between Na and CVD. Studies based on general population recruitment that excluded participants with disease at baseline were designated as having the lowest risk of bias; studies using general population recruitment that included participants with disease at baseline were designated

as having an intermediate risk of bias; and studies that specifically recruited sick participants with diseases such as congestive heart failure, end-stage renal disease, or type 1 and type 2 diabetes mellitus were designated as having a high risk of bias. Conducting sensitivity analyses for reverse causality by excluding known sick individuals or events at the beginning of follow-up may not fully account for reverse causality.[43] As such, we considered the potential for bias to be reduced only if the authors performed the above analyses and determined that the proportional hazards assumption was not violated.[44]

Domain 2: Errors With Some Potential to Alter Direction of Association

Potential for Residual Confounding 1: Incomplete Adjustment

Bias in linking Na intake and CVD can occur from underadjustment or overadjustment for potential confounding factors. In dietary studies, adjustment for calories may correct for some of the systematic error from inaccurate reporting of food.[35,45] In urine studies, adjustment for body weight (or body mass index) or creatinine excretion serves the same purpose. Confounding can be reduced by adjustment for major CVD risk factors (body mass index, cholesterol, diabetes mellitus status), demographic characteristics (age, sex, race, and socioeconomic status), and treatment status in observational analyses nested in clinical trials. We classified studies as being incompletely adjusted when these adjustment variables were not included in regression models (Table 1).

Two variables, potassium and BP, were not included in our criteria for classification. Potassium intake is highly correlated with both Na intake and CVD risk and may modify the relationship between Na intake and CVD. When potassium and Na are measured by the same method (ie, urine collection), the correlation of their errors complicates interpretation of coefficients in linear models.[37,46] A priori, we had planned to consider studies that adjusted for BP to be overadjusted because BP is likely to be an intermediary variable between Na intake and CVD. However, we dropped it from our criteria because adjustment for BP had no apparent impact on the results in several studies.[20,47–49]

Potential for Residual Confounding 2: Imbalance Across Na Intake Levels

Residual confounding can also be caused by large differences in key confounders (eg, age, sex, race) across exposure categories. In some studies, there are major sociodemographic differences between those reported to consume lower and higher levels of dietary Na that traditional regression methods may not rebalance adequately. We assessed whether the highest and lowest Na intake groups in a study differed by (1) >5 years of age, (2) >20% in the proportion of men and women, or (3) >20% in the proportion of blacks, whites, or other race/ethnic groups. We classified studies that met ≥1 of the above criteria as being at risk for residual confounding if they did not conduct a stratified analysis on the variable in question.

Inadequate Follow-up

Failure to conduct complete, high-quality follow-up of study participants can also bias results in either direction.[50] We classified studies with >20% loss to follow-up as having the potential for bias because of possible differences in outcomes between those who dropped out and those who remained under observation throughout the period of follow-up.

Domain 3: Errors With the Potential to Lead to a False Null Result

Random Error in Na Assessment

High levels of random error in estimating usual Na intake can limit the ability to assess the relationship between Na intake and disease by biasing results toward the null.[23,35] Error caused by the high day-to-day variability in Na consumption does not bias the overall mean intake because it can be assumed to be random.[35] We classified studies into 3 groups (high, intermediate, or low) based on their likely level of random error in assessing Na intake. Studies that relied on spot or overnight urines, a single 24-hour dietary recall or urine collection, or a 1-day food record were classified as having a high level of random error. Studies with ≥2 days of food records, 24-hour recalls, or two to four 24-hour urine collections or that used a second measurement on a subset of participants to estimate usual intake were considered to have intermediate levels of random error. Studies with an average of greater than four 24-hour urine measurements were considered to have low potential for random error, as were FFQs. The potential error in an FFQ is more likely systematic than random, because repeating the FFQ will not improve the validity of the assessment.

Insufficient Power

We assessed whether studies were adequately powered to detect a relationship between Na intake and CVD. To simplify the assessment, we applied a standard test: Did the study have 80% power to detect a 10% difference in CVD risk per standard deviation of Na intake? We used Stata 12.1 (StataCorp, College Station, TX) for these calculations, using the study's sample size and the CVD outcome with the largest number of events. When CVD events were not assessed, we substituted all-cause mortality; in these cases, power is likely overstated, because the expected relationship between Na intake and all-cause mortality is less than with CVD. If an article only reported subgroup analyses, we conducted a power calculation for each subgroup.

Literature Review and Data Abstraction

We attempted to identify all observational cohort studies with ≥1 year of follow-up that assessed the relationship between Na intake and CVD. The inclusion criteria were as follows: (1) prospective design, including those nested in clinical trials; (2) use of a dietary method or urine analysis to assess Na intake; and (3) ≥1 of the following outcomes: all-cause mortality, CVD mortality, stroke, CHD, congestive heart failure, or myocardial infarction. To identify eligible studies, we searched both PubMed and Embase. We also reviewed the references of previous systematic reviews. Data were abstracted onto predesigned forms to identify key features of the study.

To ensure consistency in abstracting exposure and outcome data, we used the following guidelines: We abstracted all results that used either absolute Na intake, calorie-adjusted Na intake, or Na-kilocalorie ratio as the exposure estimate.

Table 2. Study List and Main Features

Author, Year	Population	No. of Subjects	Exclusions	Strata	Na Measure	Relevant Outcome (No. of Events)	Association*
General population studies							
Kagan et al, 1985[53]	Honolulu Heart Study: Hawaiian Japanese men, 46–68 y	7088	Prior CVD	None	One 24-h recall	(1) Stroke (238)	(1) 0
Tunstall-Pedoe et al, 1997[71]	Scottish Heart Health Study: Scottish, 40–59 y	11 629	None	Men and women	One 24-h urine	(1) Progressive CHD (581) (2) CHD deaths (206) (3) All deaths (591)	(1) M: 0; W: + (2) M: 0; W: 0 (3) M: 0; W: 0
Alderman et al, 1998[18]	NHANES 1: American, 25–75 y	11 346	None	None	One 24-h recall	(1) CVD mortality (1790) (2) Mortality (3923)	(1) 0 (2) –
He et al, 1999[17]	NHANES 1: American, 25–74 y	9485	Prior CV events and low-salt diet	Normal weight and overweight	One 24-h recall	(1) Stroke (680) (2) Stroke mortality (210) (3) CHD (1727) (4) CHD mortality (614) (5) CVD mortality (895) (6) Total mortality (2486)	(1) N: 0; O: + (2) N: 0; O: + (3) N: 0; O: 0 (4) N: 0; O: + (5) N: 0; O: + (6) N: 0; O: +
Tuomilehto et al, 2001[60]	Finnish, 25–64 y	2436	Prior CV events	None†	One 24-h urine	(1) Stroke (84) (2) CHD (128) (3) CHD mortality (61) (4) CVD mortality (87) (5) Total mortality (180)	(1) 0 (2) + (3) + (4) + (5) +
He et al, 2002[52]	NHANES I: American, 25–74 y	10 362	History of CHF and low-salt diet	Normal weight and overweight	One 24-h recall	(1) CHF (1092)	(1) N: 0; O: +
Nagata et al, 2004[56]	The Takayama Study, Japanese, ≥35 y	29 099	Prior CVD or cancer	Men and women	FFQ	(1) Stroke mortality (269)	(1) M: +; W: 0
Cohen et al, 2006[67]	NHANES II: American, 30–74 y	7154	Prior CVD and low-salt diet	None	One 24-h recall	(1) Stroke mortality (79) (2) CHD mortality (282) (3) CVD mortality (541) (4) All-cause mortality (1343)	(1) 0 (2) – (3) – (4) 0
Geleijnse et al, 2007[62]	Rotterdam study: Dutch, ≥55 y	1448‡	None	None	1 Overnight urine	(1) Stroke (181) (2) MI (206) (3) CVD mortality (217) (4) Total mortality (795)	(1) 0 (2) 0 (3) 0 (4) 0
Cohen et al, 2008[19]	NHANES III: American, ≥30 y	8699	Prior CV events and low-salt diet	None	One 24-h recall	(1) CVD mortality (436) (2) All-cause mortality (1150)	(1) 0 (2) –
Umesawa et al, 2008[61]	JACC: Japan, 40–79 y	58 780	Prior CVD and cancer	None	FFQ	(1) Stroke mortality (986) (2) CHD mortality (424) (3) Total CVD mortality (1410)	(1) + (2) 0 (3) +
Cook et al, 2009[66]	TOHP 1 and 2: American, 30–54 y, prehypertensive, overweight (TOHP 2)	2974	HTN medications, prior CV events	None	One to seven 24-h urine collections	(1) CVD events (193)	(1) 0
Takachi et al, 2010[58]	JPHC: Japan, 40–69 y	70 421	Prior CV events and cancer	None	FFQ	(1) Stroke (1745) (2) MI (338) (3) CVD (2066)	(1) + (2) 0 (3) +
Stolarz-Skrzypek et al, 2011[47]	FLEMENGHO and EPOGH cohorts: European, ≥20 y	3681	Prior CVD	None	One 24-h urine	(1) Stroke (33) (2) Coronary events (98) (3) All CVD events (232) (4) CVD mortality (84) (5) All-cause mortality (219)	(1) 0 (2) 0 (3) 0 (4) – (5) 0
Yang et al, 2011[20]	NHANES III: American, ≥20 y	12 267	Prior CV events and low-salt diet	None	Usual intake, one to two 24-h recalls	(1) CHD mortality (433) (2) CVD mortality (825) (3) All-cause mortality (2270)	(1) 0 (2) 0 (3) +
Gardener et al, 2012[63]	Northern Manhattan Study: American, >40 y	2657	Prior stroke or MI	None	FFQ	(1) Stroke (235) (2) MI (209) (3) CVD event (615) (4) CVD mortality (371)	(1) + (2) 0 (3) + (4) 0

(*continued*)

Table 2. Continued

Author, Year	Population	No. of Subjects	Exclusions	Strata	Na Measure	Relevant Outcome (n)	Association*
Special population studies							
Alderman et al, 1995[69]	American, workplace-based hypertensive cohort, advised to avoid high-Na food	2937	None	Men and women	One 24-h urine	(1) MI (55) (2) All CVD (117) (3) Stroke (23)	(1) M: –§ (2) NA (3) NA
Larsson et al, 2008[48]	ATBC: Finnish male smokers, 50–69 y	26 556	Prior stroke or cancer	None	FFQ	(1) Cerebral infarction (2702) (2) Intracerebral hemorrhage (383) (3) Subarachnoid hemorrhage (196)	(1) 0 (2) 0 (3) 0
Dong et al, 2010[65]	Chinese, peritoneal dialysis	305	None	None	Multiple 3-d food records	(1) CVD mortality (32) (2) Total mortality (74)	(1) – (2) –
Thomas et al, 2011[59]	FinnDiane cohort: Finnish, T1DM	2807	ESRD	None	One 24-h urine	(1) All-cause mortality (217)	(1) J
O'Donnell et al, 2011[49]	ONTARGET and TRANSCEND trials: International, ≥55 y, CVD or T2DM	28 880	Serious valvular disease, SBP >160 mm Hg, serious CKD or CHF	None	1 Morning urine	(1) Stroke (1282) (2) MI (1412) (3) CVD events and CHF (4729) (4) CVD mortality (2057) (5) Total mortality (3430) (6) CHF (1213)	(1) 0‖ (2) 0‖ (3) J (4) J (5) 0‖ (6) 0‖
Son et al, 2011[57]	South Korean, heart failure	232	MI, stroke in past 6 mo, serious comorbidities	None	One 24-h urine	(1) Any cardiac-related event (101)	(1) +
Arcand et al, 2011[68]	Canadian, heart failure, 18–85 y	123	CKD	None	Two 3-d food records	(1) Acute decompensated heart failure (73) (2) All-cause mortality/ transplantation (30)	(1) + (2) +
Ekinci et al, 2011[64]	Australian, T2DM	638	None	None	One to five 24-h urine	(1) CVD mortality (75) (2) Total mortality (175)	(1) – (2) –
Lennie et al, 2011[54]	American, heart failure	302	ESRD, MI, stroke in past 3 mo, other terminal illness	NYHA class I/II and class III/IV	One 24-h urine	(1) Any cardiac-related event (77)	(1) I/II: – III/IV: +
McCausland et al, 2012[55]	HEMO Study: American, hemodialysis, 18–80 y	1770	Other end-stage comorbid conditions	None	Two 24-h recalls	(1) All-cause mortality (750)	(1) +

ATBC indicates Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CHD, coronary heart disease; CHF, congestive heart failure; CKD, chronic kidney disease; CV, cardiovascular; CVD, cardiovascular disease; EPOGH, European Project on Genes in Hypertension; ESRD, end-stage renal disease; FFQ, food frequency questionnaire; FLAMENGHO, Flemish Study on Environment, Genes and Health Outcomes; HEMO, hemodialysis; HTN, hypertension; J, J-shaped association; JACC, Japan Collaborative Cohort Study for the Evaluation of Cancer Risks; JPHC, Japan Public Health Center-Based Prospective Study; M, men; MI, myocardial infarction; N, normal weight; NA, not applicable; Na, sodium; NHANES, National Health and Nutrition Examination Survey; NYHA, New York Heart Association; O, overweight; ONTARGET, Ongoing Telmisartan Alone and in Combination With Ramipril Global Endpoint Trial; SBP, systolic blood pressure; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TOHP, Trials of Hypertension Prevention; TRANSCEND, Telmisartan Randomized Assessment Study in ACE Intolerant Subjects With Cardiovascular Disease; and W, women.

*Associations listed are from fully adjusted models and use the studies' designated main Na intake variable: Na intake, Na/calories, or calorie-adjusted Na residuals. Listed as significant if either the linear trend was significant or there was a significant difference between the highest and lowest Na intake groups. 0 indicates null; +, positive significance; and –, negative significance.

† Presented in strata by sex and combined.

‡ Number in subcohort (case cohort study).

§In men only; only crude associations were calculated for women and other outcomes.

‖Showed positive significant results between middle and top of distribution, but overall J shape was not significant.

When >1 measure of Na intake was reported, the choice of study results and resultant domain classification was based on our perception of the article's main exposure, typically the one highlighted in the article's abstract. We abstracted results for continuous, categorical, and nonlinear Na intake measures. We reported the results for all outcomes that met our inclusion criteria, with the exception of stroke subtypes. These were only reported where total stroke was unavailable.

Using the fully adjusted model, we categorized studies according to whether or not they showed a direct, inverse, J-shaped, or null relationship between Na intake and CVD. We reported subgroup-specific results when the overall results were not published (henceforth called *substudies*). A study or substudy was considered to have a positive association if ≥1 of the exposure-outcome relationships showed a significant positive association and the remainder were null. The same principle was applied to identification of inverse and J-shaped relationships. Each exposure-outcome relationship was not considered an independent finding. A study was considered to show a null relationship only if all of the results were nonsignificant. We considered results to be significant if the *P* value for the Na intake and CVD relationship was <0.05. J-shaped relationships were assessed only if the authors specifically tested for them (3 studies).

Results

Our literature search identified 3487 publications, of which 81 met ≥1 of the inclusion criteria. A total of 26 articles met all of our criteria for inclusion in the present analysis,[17–20,47–49,52–70] but 1 duplicate publication was excluded.[70] One additional report was identified from a prior systematic review,[16,71] which resulted in 26 articles.

The 26 included articles reported results for a total of 31 independent analyses conducted in 285 530 participants. Three articles used data from the National Health and Nutrition Examination Survey (NHANES) I,[17,18,52] and 2 used NHANES III data.[19,20] Participants were from East Asia, North America, Europe, and Australia. Sixteen articles were based on general population recruitment and 10 on recruitment of participants at elevated risk of CVD (Table 2). Individual studies assessed the relationship between Na and 1 to 6 clinical CVD outcomes. Nine studies assessed the relationship with stroke incidence and 4 with stroke mortality; 9 assessed CHD or myocardial infarction incidence, and 6 assessed CHD mortality; 5 assessed any CVD incidence, and 13 assessed any CVD mortality; 1 assessed congestive heart failure incidence, and 4 assessed congestive heart failure–related hospitalization or mortality; and 15 assessed all-cause mortality. Definitions of CVD were inconsistent across studies.

Within the 31 independent analyses, results varied across clinical outcomes, but no study or substudy reported a positive significant finding for 1 outcome and an inverse or J-shaped significant finding for another. Although null findings were the most common (reported for ≥1 outcome in more than half the studies surveyed), only 8 studies or substudies reported solely null associations. Overall, there was a significant, positive association between Na and ≥1 outcome in 13 of the studies or substudies, at least 1 significant inverse association in 8 and J-shaped associations in 2. Findings for stroke (7 null, 3 positive), stroke mortality (3 null, 3 positive), and CHD mortality (6 null, 2 positive) were the most consistent, with no inverse associations. Findings for CHD incidence (8 null, 2 positive, 1 inverse), CVD incidence (2 null, 2 positive, 1 inverse), CVD mortality (5 null, 3 positive, 6 inverse), and all-cause mortality (8 null, 5 positive, 4 inverse) were more mixed (Table 2; online-only Data Supplement Table 1).

Domain 1: Errors With the Greatest Potential to Alter the Direction of Association

Systematic Error in Na Assessment

Na intake was assessed by means of urine collections in 11 studies, 9 of which used at least one 24-hour urine collection. In the remaining 15 studies, dietary methods were used to assess Na intake, with 10 using 24-hour recalls or food records and 5 using FFQs. Of the 9 studies that used 24-hour urine collection, 6 reported some quality assurance procedures or excluded incomplete collection. Of these 5, 1 measured Na after participants were asked to reduce their Na intake[69] and 1 provided data documenting systematic error,[47] and thus, only 4 were classified as having a lower risk of systematic error. One of the studies that used food records also asked participants to alter their diet to facilitate measurement of Na intake.[65]

Although most studies did not provide the level of information required to assess whether systematic error was present, we identified evidence of it in 1 study that used 24-hour urine collections and in 5 that used dietary surveys. One study showed evidence of undercollection of 24-hour urine samples: In men, creatinine excretion levels differed by 24.8% between the lowest and highest tertiles of Na intake, whereas weight differed by just 9.8% in these tertiles[47] (Figure; online-only Data Supplement Table 2a). The Figure also provides an example typical of the 5 dietary studies with observed systematic error: Calorie intake differed by 49.8% between men in the lowest and highest quartile of Na intake, whereas the corresponding difference in weight was only 2.2%[18] (online-only Data Supplement Table 2b).

Potential for Reverse Causality

Seven studies that recruited participants with congestive heart failure, end-stage renal disease, type 1 and type 2 diabetes mellitus, or prior CVD were classified as having a high potential for reverse causality. Another 7 studies did not exclude sick participants and thus were classified as having an intermediate level of risk (number of included participants with known prior CVD ranged from 2%–21%). Two additional studies were assessed as having intermediate risk: 1 excluded sick participants at baseline, but 18% still had evidence of previous cardiac disease[63]; another recruited sick participants but met our criteria for testing for reverse causation.[49] The remaining 10 studies were judged to have a low potential for reverse causality, recruiting general samples and excluding participants with known prior CVD (Table 3; online-only Data Supplement Table 3).

Domain 2: Errors With Some Potential to Alter the Direction of Association

Potential for Residual Confounding 1: Incomplete Adjustment

More than half (14) of the studies had a potential risk of bias because of underadjustment. Of these, 2 controlled for age and sex only; 7 used a urinary assessment of Na intake but did not control for creatinine excretion or weight (or body mass index); and an additional 5 did not control for ≥2 traditional CVD risk factors or demographic variables (Table 3; online-only Data Supplement Table 4a).

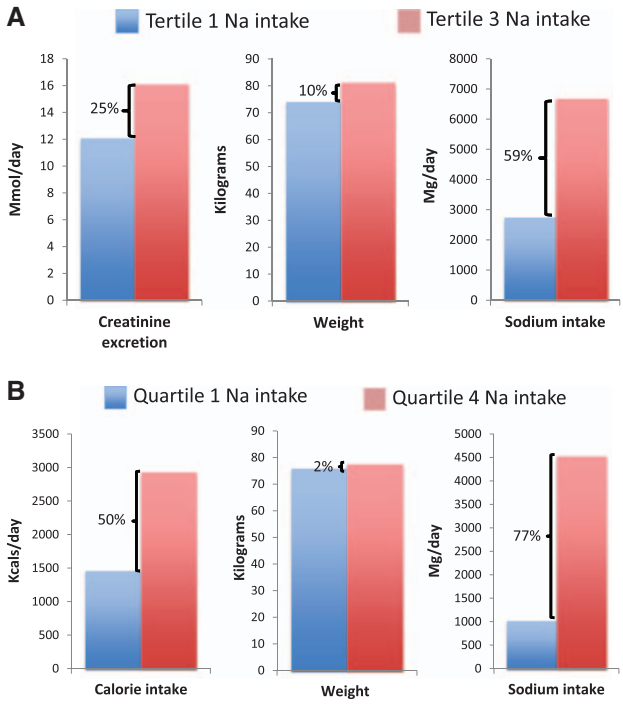

Figure. Systematic error in sodium (Na) assessment in men. Data derived from Stolarz-Skrzypek et al[47] (**A**) and Alderman et al[18] (**B**).

Potential for Residual Confounding 2: Imbalance Across Groups

Eight studies were deemed to lack balance across categories of Na intake. Two studies had a >5-year age difference between the highest and lowest Na intake groups, 4 had a >20 percentage point difference in the percentage of men across Na intake groups, and another 3 met both criteria. In 4 of the studies identified above, analyses stratified by the relevant confounder indicated a low risk of residual confounding. The potential for residual confounding was deemed low in 9 studies and could not be assessed in another 9 studies because of lack of information (Table 3; online-only Data Supplement Table 4b).

Inadequate Follow-up

Two studies had a >20% loss to follow-up (for nonfatal outcomes), and 4 studies did not report the completeness of follow-up (Table 3; online-only Data Supplement Table 5). Follow-up quality was generally high, with most studies reporting that cases were confirmed with medical records; however, 9 studies relied solely on data from death registries to classify the cause of death.

Domain 3: Errors With the Potential to Lead to a False Null Result

Random Error in Na Assessment

Assessment of random error depended on both the type and frequency of Na measurements. Of the 11 studies that used urine collections to assess Na intake, only 2 used multiple urine collections, thus reducing the likelihood of random error: Ekinci et al[64] used one to five 24-hour collections, and Cook et al used 3 to 7 collections.[66] Of the 10 studies that used 24-hour recall or food records to assess Na intake, 4 assessed >1 day of intake (including 1 that used a second day of intake from a subset of participants to estimate usual intake)[20] and had an intermediate likelihood of random error. Five studies used FFQs to assess Na intake and were designated as having low potential for random error (Table 3; online-only Data Supplement Tables 2a and 2b).

Insufficient Power

Among the 30 studies or sub-studies in which statistical power could be assessed, 8 met our threshold of 80% power to detect a 10% difference in CVD risk. Power was <50% in 15 sub-studies (Table 3; online-only Data Supplement Table 5).

Methodological Challenges by Direction of Association

On average, we identified 3 to 4 methodological challenges in each of the 31 studies or substudies. Methodological challenges were evident no matter the direction of the association between Na and CVD. Those in domain 1 (errors with the greatest potential to alter the direction of association) were approximately evenly distributed regardless of the direction of the association, although they were slightly more common in studies with an inverse and J-shaped association than in studies with a positive association (7/10 versus 7/13; potential for systematic error 9/10 versus 10/13). Domain 2 (errors with lower potential to alter the direction of association) followed a similar pattern (7/10 versus 7/13 for inadequate adjustment; 5/10 versus 4/6 for imbalance across groups; 1/9 versus 0/12 for inadequate follow-up). Errors in domain 3 (those likely to lead to a false null result) were the most common overall and were found in all but 3 studies (Table 3). Of the null studies, 63% (5/8) had high levels of random error, and 71% (5/7) had <80% power.

Discussion

The present study has 2 main findings. First, methodological challenges were common across all of the assessed domains. Errors with a potential to alter the direction of the association in either direction (domains 1 and 2) were common across all studies and slightly more prevalent in studies that reported an inverse or J-shaped relationship. Errors likely to lead to a false null result (domain 3) were also common, perhaps accounting for the preponderance of null outcomes. Second, many of the reports provided insufficient information to assess study quality. This was particularly true for assessment of systematic errors in Na intake.

The potential for systematic error in Na assessment is a major concern. One way to partially reduce the systematic error from underreporting of foods in dietary studies is to correct for energy intake; however, this technique does not eliminate error attributable to inaccurate food composition tables or failure to include discretionary salt use in the assessment. It also means assessing Na indexed to calories rather than absolute Na levels, the approach used in the current dietary recommendations for Na of 1500 or 2300 mg of Na per day regardless of calorie intake.[72] Standardizing to creatinine excretion in 24-hour urine collections can also reduce systematic error that arises through undercollection, although this was rarely done in the studies we assessed.

The use of more than one 24-hour urine collection should remain the "gold standard" for measurement of individual

Table 3. Characteristics of Studies, by Direction of Association

Article	Subgroup	Domain 1: Potential for Systematic Error in Na Assessment	Domain 1: Potential for Reverse Causality	Domain 2: Inadequate Adjustment	Domain 2: Imbalance Across Groups	Domain 2: Follow-up <80%	Domain 3: Random Error in Na Assessment	Domain 3: Power <80%
Direct association		10 (77%)*	4 (31%)	7 (54%)	3/7 (43%)	0 (0%)	6 (46%)	9 (69%)
He et al, 1999[17]	Overweight							
He et al, 2002[52]	Overweight							
Yang et al[20]	All							
Tuomilehto et al[60]	All							
Tunstall-Pedoe et al[71]	Women							
Gardener et al[63]	All	†						
Takachi et al[58]	All							
Umesawa et al[61]	All							
Nagata et al[56]	Men							
McCausland et al[55]	All							
Arcand et al[68]	All	†						
Son et al[57]	All							
Lennie et al[54]	Class III/IV CHF							
Inverse association		6 (75%)	3 (38%)	6 (75%)	4 (50%)	1/8 (13%)	6 (75%)	7 (88%)
Alderman et al, 1998[18]	All	†						
Cohen et al, 2006[67]	All	†						
Cohen et al, 2008[19]	All	†						
Alderman et al, 1995[69]	Men							
Stolarz-Skrzypek et al[47‡]	All	†						
Dong et al[65]	All							
Lennie et al[54]	Class I/II CHF							
Ekinci et al[64]	All							
J-shaped association		2 (100%)	1 (50%)	1 (50%)	1 (50%)	1 (100%)	2 (100%)	1 (50%)
Thomas et al[59]	All							
O'Donnell et al[49]	All							
Null association		8 (100%)	0 (0%)	2 (25%)	1/3 (33%)	1/6 (17%)	5 (63%)	5/7 (71%)
He et al, 2002[17]	Normal weight							
He et al, 2002[52]	Normal weight							
Tunstall-Pedoe et al[71]	Men							
Geleijnse et al[62]	All							
Cook et al[66]	All							
Nagata et al[56]	Women							
Kagan et al[53]	Men							
Larsson et al[48]	Men							

CHF indicates congestive heart failure; and Na, sodium.
*Indicates the number and percent of studies in domain (with available data) classified as having a high level of potential bias.
†Indicates evidence of systematic error in Na assessment.
‡Follow up applies to non-mortality outcomes only.
Legend:
High level of potential bias
Moderate level of potential bias
Low level of potential bias
No information

level Na intake in general population studies. Nonetheless, 24-hour urinary Na measurements are prone to underestimate Na intake because of incomplete collection unless specific measures are taken to prevent this problem.[26,27] Although we gave studies credit for any quality assurance measure, to obtain an unbiased and complete assessment, investigators need to

follow procedures such as asking participants to begin and end collections in the clinic and applying rigorous quality control procedures similar to those implemented in the INTERMAP study (International Study of Macro- and Micro-Nutrients and Blood Pressure; Appendix A, Manual of Operations).[26] Most of the included articles used urine samples that had either been collected in studies in which assessment of Na relationships was not the primary goal or as part of routine clinical practice. When quality assurance information related to urine collection was provided, it was often quite limited. Only 5 of the 9 included studies reported quality assurance procedures or excluded participants on the basis of incomplete collections,[47,54,57,60,66] and 1 excluded incomplete collections in a sensitivity analysis.[69] Exclusions in these studies ranged from <1% to 11% compared with the 20% to 25% reported in studies that used PABA (p-aminobenzoic acid) testing[28] (online-only Data Supplement Table 2a).

Of the 26 articles, 12 were published after 2010. These recent articles more often recruited sick study participants (8/12). It is important to understand the relationship between Na intake and CVD in sick patients because it may differ from the relationship in the general population; however, such findings are not directly relevant to recommendations for the general population. Differences in findings between general population cohorts and studies with a high prevalence of sick patients may also be related to reverse causality in the latter group. This is a particularly relevant concern in studies of mortality outcomes,[43] which are frequently of interest in investigations conducted in sick patients. In addition, valid estimation of 24-hour urinary Na can be challenging in this setting.[73]

The impact of methodological choices on study findings is demonstrated by the striking differences in results from separate analyses of the same study in which investigators used different inclusion criteria and analytic approaches. He et al[17] reported a positive significant association between Na intake and all-cause mortality in overweight NHANES I participants and a null association in normal-weight participants. In contrast, Alderman et al[18] reported a significant inverse association between absolute levels of Na intake and all-cause mortality in all participants. In addition to the use of subgroup analyses in the study by He et al,[17] differences included the following: (1) Alderman et al[18] did not exclude participants with CVD or those on a low-salt diet to reduce their blood pressure (≈13%); and (2) Alderman et al[18] used models that did not control for key CVD risk factors but included the Na-kilocalorie ratio, Na intake, and kilocalories simultaneously in the model. In an analysis of NHANES III, Cohen et al[19] found a significant inverse relationship linking Na intake to CVD mortality (null for all-cause mortality). In contrast, an exploration of the same data set by Yang et al[20] identified a significant positive relationship between Na intake and all-cause mortality (null for CVD mortality). The main differences between the 2 studies were that Yang et al[20] used longer follow-up and used estimated usual Na intake rather than a single dietary recall. When Yang et al[20] used a single dietary recall, the results for all-cause mortality were null and thus similar to those reported by Cohen et al[19] (online-only Data Supplement Table 6).

The present review has several strengths. Although meta-analyses of observational studies linking Na intake and clinical cardiovascular outcomes have been conducted, this is the first systematic review focusing specifically on the quality of these studies. We identified 3 principal domains that encompass the potential for systematic and random error. For each domain, we defined objective standards and applied them consistently to all studies. Generic quality metrics (eg, GRADE [Grading of Recommendations Assessment, Development and Evaluation][74] or the Downs and Black checklist[75]) lack specific guidance on how to assess bias, particularly as it relates to nutrition-specific issues. Furthermore, some automatically downgrade observational studies regardless of their quality. Finally, studies with a single major flaw that leads to systematic bias may nonetheless receive an overall high score based on other criteria.

The present review also has limitations. As with any classification system, the results are not purely quantitative and required judgments by the study team. However, to the extent possible, we applied uniform standards to the identification of potential errors in each study. In some cases, a customized study-specific approach to managing and avoiding error may be more appropriate. For instance, it may be prudent to control for different variables in studies of dialysis patients compared with the general population. Second, in many of the reports, there was insufficient information to assess the potential for methodological flaws, particularly in terms of assessing the presence of systematic errors in Na measurement. Finally, we did not assess the effect on type 1 error given the large number of outcomes, subgroups, and analytic approaches in many studies.

The present analysis can serve as a resource for investigators during the design, conduct, and analysis of future studies. Studies in general population samples, that is, broadly inclusive studies that are not restricted to individuals with a specific disease or condition, have the potential to provide the most valuable information, both because of their larger policy implications and their enhanced potential to avoid reverse causality. Furthermore, studies should focus on CVD incidence, if possible, because this outcome is less prone to be influenced by reverse causality. Another important lesson from the existing reports is that Na measurement needs more careful attention during study design and conduct, not simply during the analysis. Collection of multiple complete 24-hour urine specimens is a substantial burden for study participants, but there is, as yet, no satisfactory substitute. Additional research on the validity of overnight, spot, and timed urine collections may be useful, but diurnal variation in Na excretion makes it unlikely that they can serve as a satisfactory substitute for 24-hour collections at the individual level. Finally, the present study highlights the need for more complete reporting by authors so that reviewers and readers can assess the completeness of urine collections and evaluate other potential sources of error. Body weight (or body mass index) and urine creatinine excretion should be reported by categories of Na intake in studies that use 24-hour urine collections to assess Na intake, whereas total calorie intake should be reported in studies that use a dietary collection method.

The present study can also help researchers and policy makers interpret the results of existing and future studies of the relationship between Na intake and CVD. Methodological issues have the potential to qualitatively

affect the results and interpretation of studies. The present study has shown that flaws are common in the studies that have been conducted to date, especially those conducted in sick individuals. However, not all studies are flawed to the same extent. Studies with the lowest risk of reverse causality and systematic error in exposure assessment are likely to be the least biased. In general, we recommend reliance on studies conducted in the general population that used 24-hour urine collections with available quality assurance. Although recent meta-analyses of this body of literature have been useful in summarizing the relationship between Na and CVD outcomes, novel methods that allow for classification of studies and then weighting of them by likely level of bias could improve the validity of results.

Overall, however, we do not recommend using this body of literature to set specific cut points for Na intake recommendations, as a few recent reports have done. Using the literature reviewed in the present report plus a few relevant trials, the Institute of Medicine found that the evidence for the current US Dietary Guidelines 2300 mg/d recommendation was compelling but that data for limiting intake to 1500 mg/d in subgroups were insufficient.[76] A recent article by O'Donnell et al,[77] also using the same body of literature, suggested an even higher threshold for healthy Na intake. However, both correctly point out that given the multiplicity of different measures of intake and the lack of standardization, it is difficult to make comparisons across studies to determine an optimal level of intake. For the foreseeable future, the high-quality body of evidence linking Na intake to BP[2] should remain the basis for setting recommended levels of Na intake.

In conclusion, it is difficult to conduct rigorous, high-quality investigations of the relationship between Na intake and CVD. Most of the available information on this topic has been derived from secondary analyses of studies that were not designed to answer this question. There is a high likelihood that similar additional reports will be published and may suffer from the same biases and methodological flaws highlighted here. We hope that the present report will provide a blueprint to gauge the quality of these studies and to ensure that Na policies are based on the best data available.

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This table represents the relationships of writing group members that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all members of the writing group are required to complete and submit. A relationship is considered to be "significant" if (1) the person receives $10 000 or more during any 12-mo period, or 5% or more of the person's gross income; or (2) the person owns 5% or more of the voting stock or share of the entity, or owns $10 000 or more of the fair market value of the entity. A relationship is considered to be "modest" if it is less than "significant" under the preceding definition.

*Modest.

†Significant.

Reviewer Disclosures

Reviewer	Employment	Research Grant	Other Research Support	Speakers' Bureau/ Honoraria	Expert Witness	Ownership Interest	Consultant/Advisory Board	Other
Francesco Cappuccio	University of Warwick, United Kingdom	BUPA Foundation (money goes to institution)†	None	None	None	None	World Health Organization (unpaid)*; National Institute of Health & Clinical Excellence (unpaid)*	None
Darwin R. Labarthe	Northwestern University	None	None	None	None	None	None	None
Lyn Steffen	University of Minnesota	None	None	None	None	None	None	None
Pasquale Strazzullo	University of Naples, Italy	PI of (1) the MINISAL-GIRCSI study and (2) the "Lower Salt Community trial." Both grants provided by the Italian Ministry of Health†	None	None	None	None	World Action on Salt and Health (WASH) (unpaid)*; Coordinator of the Interdisciplinary Working Group for Reduction of Salt Intake in Italy (GIRCSI), a member of the SINU/INRAN Committee for the preparation of the Italian Dietary Reference Intakes (unpaid)*	None

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*Modest.

†Significant.

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Supplementary Table 1: Relationship between sodium (Na) intake and clinical cardiovascular outcomes (higher versus lower Na intake)

First author	Na metric	Strata	All stroke	Stroke mortality	All coronary heart disease	Coronary heart disease mortality	All cardiovascular disease	Cardiovascular disease mortality	All cause mortality	Congestive heart failure
General Population with Diet-Based Na Measures										
Alderman, 1998[18]	per SD	All						0.89 (0.77 - 1.02)	**0.88 (0.8 - 0.96)**	
He, 1999[17]	100 mmol/7452 kJ	Normal weight	0.98 (0.83-1.16)	0.90 (0.63 - 1.28)	0.95 (0.83 - 1.10)	1.07 (0.87 - 1.31)		1.02 (0.85 - 1.22)	1.00 (0.90 - 1.11)	
	100 mmol/7452 kJ	Overweight	**1.32 (1.07 - 1.64)**	**1.89 (1.31 - 2.74)**	1.06 (0.88 - 1.29)	**1.44 (1.14 - 1.81)**		**1.61 (1.32 - 1.96)**	**1.39 (1.23 - 1.58)**	
He, 2002[52]	per 100 mmol	Normal weight								0.90 (0.67 - 1.20)
	per 100 mmol	Overweight								**1.26 (1.03 - 1.53)**
Cohen, 2006[67]	per 1000mg	All		0.95 (0.75 - 1.21)		0.91 (0.79 - 1.05)		**0.89 (0.80 - 0.99)**	0.93 (0.87 - 1.00)*	
Cohen, 2008[19]	Q4 v. Q1	All						**0.56 (.32 - .95)**	0.81 (.59 - 1.10)	
Yang[20]	per 1000 mg	All				1.20 (0.81 - 1.77)		0.94 (0.67 - 1.32)	**1.2 (1.03 - 1.41)**	
Kagan[53]	Q5 v Q1	All	p = .348 (HR not shown)							
Nagata[56]	T3 v T1	Men		**2.33 (1.23 - 4.45)**						
	T3 v T1	Women		1.70 (0.96 - 3.02)						
Umesawa[61]	Q5 v Q1	All		**1.55 (1.21 - 2.00)**		1.19 (0.82 - 1.73)		**1.42 (1.20 - 1.69)**		
Takachi[58]	Q5 v Q1	All	**1.21 (1.01 - 1.43)**		1.09 (0.71 - 1.68)[a]		**1.19 (1.01 - 1.40)**			
Gardener[63]	per 500 mg	All	**1.17 (1.07 - 1.27)**		0.94 (0.85 - 1.04)		**1.05 (0.99 -1.11)***	1.02 (0.95 - 1.1)		
General Population with Urine Collection Based Na measures										
Tunstall-Pedoe[71]	per 1/5 change	Men			1.05 (0.96 - 1.14)	0.98 (0.86 - 1.13)			0.92 (0.84 - 1.00)	
	per 1/5 change	Women			**1.16 (1.00 - 1.13)**	1.14 (0.87 - 1.49)			0.97 (0.86 - 1.10)	
Tuomilehto[60]	per 100 mmol	Both sexes	1.13 (0.84 - 1.51)		**1.34 (1.08 - 1.67)**	**1.56 (1.15 - 2.12)**		**1.36 (1.05 - 1.76)**	**1.22 (1.02 - 1.47)**	
Geleijnse[62]	per SD	All	1.08 (0.80 - 1.46)		1.19 (0.97 - 1.46)[a]			0.77 (0.60 - 1.01)	0.95 (0.81 - 1.12)	
Cook[66]	Q4 v. Q1	All					1.42 (0.99 - 2.04)			
Stolarz-Skrzypek[47]	compared to overall mean	All	T1: 1.07 (0.57 - 2.00) T3: 0.78 (0.45 - 1.33)		T1: 1.42 (0.99 - 2.04) T3: 0.86 (0.65 - 1.13)		T1:1.13 (0.90 - 1.42) T3: 0.90 (0.73 - 1.11)	**T1: 1.56 (1.02 - 2.36) T3: 0.95 (0.66 - 1.38)**	T1: 1.14 (0.87 - 1.50) T3: 1.06 (0.84 - 1.33)	
Special Population with Diet-Based Na Measures										
Larsson[48]	Q5 v Q1	All	1.04 (0.91 - 1.18)[d]							
Dong[65] **	per 1000 mg	All						**0.11 (0.03 - 0.48)**	**0.44 (0.20 - 0.95)**	
Arcand[68]	T3 v. T1	All							**3.54 (1.46 - 8.62)**	**2.55 (1.61 - 4.04)[c]**
McCausland[55]	per mg/kcal	All							**~1.2**	
Special Population with Urine Colleciton Based Na Measures										
Alderman, 1995[69]***	per SD change	men			**0.68 (0.46 - 0.99)**					
Son[57]	>3 g v. <3 g	All								**1.81 (1.17 - 2.80)[b]**
Thomas[59]	non-linear	All							**J (p <0.001)**	
Lennie[54]	>3 g v. <3 g	Class I/II CHF								**0.44 (0.20 - 0.97)[b]**
	>3 g v. <3 g	Class III/IV CHF								**2.54 (1.10 - 5.83)[b]**
O'Donnell[49]	Q1 v Q4 Q8 v. Q4	All	Q1: 1.06 (0.76 - 1.46) Q8: 1.48 (1.09 - 2.01)		Q1: 1.10 (0.80 - 1.53) Q8: 1.48 (1.11 - 1.98)[a]		**Q1: 1.21 (1.03 - 1.43) Q8: 1.49 (1.28 - 1.75)**	**Q1: 1.37 (1.09 - 1.73) Q8: 1.66 (1.31 - 2.10)**	Q1: 1.19 (0.99 - 1.45) Q8: 1.56 (1.30 - 1.89)	Q1: 1.29 (0.95 - 1.74) Q8: 1.51 (1.12 - 2.05)
Ekinci[64]	per 100 mmol	All						**0.65 (0.44 - 0.95)**	**0.72 (0.55 - 0.94)**	

*Additional test significant (binary, Q4 v. Q1, etc.)

** Two intake variables: baseline and average intake. Average intake used here to match primary exposure

***Fully adjusted models were provided only for MI in men;

Supplementary Table 2a: Systematic error in sodium (Na) intake in studies that use urine collection

Author	Collection type	Creatinine excretion		Weight (kg)[c] or BMI (kg/m^2)[tt]		Sodium (mg)		QA measures?	Corrected for Creatinine	Incomplete collections excluded?	Random Error?	Potential for systematic error?	Systematic error observed
		Q1	Q4	Q1	Q4	Q1	Q4						
Tunstall-Pedoe[71 c]	1 24-hr urine	Not available		M:23.3[tt] W: 22.1[tt]	M:28.7[tt] W: 28.9[tt]	M: 2981 W: 2254	M: 5780 W: 4308	Not available	**No**	Not available	High	High	Cannot rule out
Tuomilehto[60]	1 24-hr urine	Not available		M: 25.5[tt] W: 24.6	M: 28.1[tt] W: 27.8	M: <3657 W: <2737	M: >6026 W: >4462	Not available	No	Based on self-report (7%)	High	Lower	Cannot rule out
Geleijnse[62 b]	1 overnight urine	Not available		Not available		Not available		Yes	No	Based on volume and recorded collection times (9.3%)[d]	High	High	Cannot rule out
Cook[66]	3-7 24-hr urine (median: 5 in TOHP I; 4 in TOHP II)	Not available		Not available		Not available		Not available	No	Excluded 15 people for lack of any valid urine collections	Low	Lower	Cannot rule out
Stolarz-Skrzypek[47 b]	1 24-hr urine	M: 12.1 mmol W: 8.4 mmol	M: 16.1 mmol W: 10.6 mmol	M: 74.1[t] W: 63.4[t]	M: 81.2[t] W: 68.7[t]	M: 2762 W: 2187	M: 6682 W: 5329	Not available	No	Based on volume and creatinine (3%)	High	High	Yes
Alderman, 1995[69]	1 24-hr urine	Not available		79.4[t]	87.5[t]	1495	4945	Not available	No	In sensitivity analysis, based on Cockcroft & Gault formula (32%)	High	High	Yes
Son[57]	1 24-hr urine	Not available		Not available		Not available		Yes	No	Based on self report (7%)	High	Lower	Cannot rule out
Thomas[59]	1 24-hr urine	Not available		24.7[tt]	26.1[tt]	<2346	>4301	Not available	No	Not available	High	High	Cannot rule out
Lennie[54]	1 24-hr urine	Not available		Not available		Not available		Yes	No	Based on self report and concentration (11%)	High	Lower	Cannot rule out
O'Donnell[49 a]	1 fasting morning spot urine	95.55 µmol/L	93.75 µmol/L	27.3[tt]	30.2[tt]	1550	9400	N/A	Yes, part of equation.	N/A	High	High	Cannot rule out
Ekinci[64 b]	1-5 24-hr urine (median 2)	Not available		Not available		<3450	>4784	Not available	No	Not available	Intermediate	High	Cannot rule out

[a] groups are predetermined by 2000 mgs

[b] tertiles

[c] 20th and 80th percentiles

Supplementary Table 2b:Systematic error in sodium (Na) intake in studies that use dietary methods to assess Na intake

Author	Collection type	Kcals		Weight (kg) / BMI (kg/m^2)		Sodium intake (mg)		Difference in kcal	Difference in weight or BMI	Intake measure	Random error?	Potential for systematic error?	Systematic error observed?
		Q1	Q4	Q1	Q4	Q1	Q4						
Kagan[53]	1 24 hr dietary recall	Not available		Not available		Not available		N/A	N/A	Na intake	High	High	Cannot assess
Alderman, 1998[18]	1 24 hr dietary recall	M:1473 W: 989	M: 2937 W: 1976	M: 76.0 W: 68.4	M: 77.7 W: 64.3	M: 1041 W: 678	M: 4538 W: 3105	M: 46% W: 50%	M: 0.02% W: -0.06%	Na intake Na/kcal ratio (in the same model)	High	High	Yes
He, 1999[17 a]	1 24 hr dietary recall	N: 1908 O: 1723	N: 1731 O: 1546	N: 23.1 O: 32.0	N: 23.2 O: 31.6	N: 1162 O: 1047	N: 3278 O: 2983	M: -8% W: -11%	M: 0.4% W: -1.2%	*Na/kcal ratio Na intake	High	High	Cannot rule out
He, 2002[52]	1 24 hr dietary recall	Not available		Not available		N: 810 O: 775	N: 3777 O: 3855	N/A	N/A	*Na intake Na/kcal ratio	High	High	Cannot rule out
Cohen, 2006[67]	1 24 hr dietary recall	1411	2248	70.7	74.4	1579	3696	37%	5%	*Na intake Na/Kcal ratio Energy adjusted sodium intake	High	High	Yes
Cohen, 2008[19 b]	1 24 hr dietary recall	1282	2938	69.2	79.3	1501	5497	56%	13%	*Na intake Na/Kcal ratio Energy adjusted sodium intake	High	High	Yes
Yang[20]	1 24 hr dietary recall, 7% had 2 recalls	Not available		Not available		Not available		N/A	N/A	Usual Na intake, calculated by NCI method using 2 dietary recalls where available	Inter-mediate	High	Cannot rule out
Nagata[56]	169 item semi-quantitative FFQ	M: 2558 W: 2140	M: 2590 W: 2092	M: 22.6 W: 21.9	M: 22.5 W: 22.1	M: 4082 W: 3970	M: 7194 W: 6478	M: 1% W: -2%	M: - 0.4% W: 0.9%	Energy adjusted sodium intake	Low	High	Cannot rule out
Takachi[58 c]	138-item questionnaire	1959	1958	Not available		3084	6844	N/A	N/A	Energy adjusted sodium intake	Low	High	Cannot rule out
Umesawa[61 c]	35-item FFQ	1496	1466	22.8	23	2323[t]	6256	-2%	1%	Energy adjusted sodium intake	Low	High	Cannot rule out
Gardener[63 d]	Modified Block NCI FFQ	814	2413	28	29	Not available		66%	3%	Na intake	Low	High	Yes
Dong[65]	Multiple 3 day diet records[f]	1146	1469	22.6	23.7	1410	2470	22%	5%	*Average Na intake Baseline Na intake	Inter-mediate	High	Cannot rule out
Arcand[68]	2, 3 day diet records	1564	2447	28.3	30.8	1400	3800	36%	8%	Na intake	Inter-mediate	High	Yes
McCausland[5] [5]	2 diary assisted 24-hr dietary recall	Not available		Not available		Not available		N/A	N/A	Na intake *Na/kcal ratio	Inter-mediate	High	Cannot rule out
Larsson[48 c]	276 item semi-quantitative FFQ	Not available		25.8	26.8	3822	5983	N/A	N/A	Energy adjusted sodium quintiles	Low	High	Cannot rule out

[a] Na/kcal ratio groups

[b] Na intake divided into <2300/>2300 mg

[c] Na intake divided into quintiles

[d] Na intake divided into: <1500; 1501-2300; 2301-3999; >4000 mgs

[e] Na intake calibrated (values used are 2x what was reported in the FFQ)

[f] Participants specifically asked to avoid processed foods, restaurant foods or eating with their families while doing the records in order to allow them to accurately assess their sodium intake. Not clear how many assessments each

*Considered primary exposure measurement

Supplementary Table 3: Reverse causality

First author	Specifically recruited sick samples?	Includes sick population?	Percent with prior cardiovascular disease	Sensitivity analysis?	Potential for reverse causality?
General Population with Diet Based Sodium Intake Measures					
Alderman, 1998[18]	No	Yes	Men: 11-17.6% Women: 9.6 to 11.5%	Restricting to people without CVD does not change the relationship	Intermediate
He, 1999[17]	No	No, prior CVD excluded	Excluded	N/A	Low
He, 2002[52]	No	Yes, only excludes CHF at baseline	History of CHD: 4-5%; History of valvular HD: 5%	N/A	Intermediate
Cohen, 2006[67]	No	No, prior CVD and deaths in 1st 6 months excluded	Excluded	N/A	Low
Cohen, 2008[19]	No	No, prior CVD and deaths in 1st 6 months excluded	Excluded	N/A	Low
Yang[20]	No	No, prior CVD excluded	Excluded	N/A	Low
Kagan[53]	No	No, excludes prior CVD	Excluded	N/A	Low
Nagata[56]	No	No, prior stroke, IHD, and cancer excluded	Excluded	Excluding deaths in first two years strengthened positive, NS relationship for stroke in women to a significant one	Low
Umesawa[61]	No	No, prior CVD and cancer excluded	Excluded	N/A	Low
Takachi[58]	No	No, prior CVD and cancer excluded	No information	Excluding ppl treated for HTN, hyperlipidemia and diabetes strenghtened positive association	Low
Gardener[63]	No	No, prior stroke or MI excluded	Previous cardiac disease: 18%	N/A	Intermediate
General Population with Urine Collection Based Sodium Intake Measures					
Tunstall-Pedoe[71]	No	Yes	Previous CVD: 21% (W), 21.5% (M)	N/A	Intermediate
Tuomilehto[60]	No	Mortality analyses include prior CV events; excluded from incident analysis	Prior CV event: 1.6%	N/A	Intermediate
Geleijnse[62]	No	Yes	History of CVD: 17% in subcohort	Excluding people without baseline CVD or HTN did not change null results (no consistent effect)	Intermediate
Cook[66]	No	prehypertensive, not on HTN meds, second cohort overweight	Excluded	N/A	Low
Stolarz-Skrzypek[47]	No	No, prior CVD excluded	Excluded	Excluding first 3 years of follow up did not change inverse or null results	Low
Special Population with Diet Based Sodium Intake Measures					
Larsson[48]	Yes, smokers	Yes, but excluded those with stroke or "serious disease" precluding long term participation	No information	N/A	Intermediate
Dong[65]	Yes, peritoneal dialysis	Yes	100% ESRD	N/A	High
Arcand[68]	Yes, CHF	Yes	100% CHF	N/A	High
McCausland[55]	Yes, Hemodialysis	Yes	100% ESRD	N/A	High
Special Population with Urine Collection Based Na Measures					
Alderman, 1995[69]	Yes, HTN	Yes, no exclusions	CVD: 8%	N/A	Intermediate
Son[57]	Yes, CHF	Yes	100% CHF	N/A	High
Thomas[59]	Yes, T1DM	Yes	Macrovascular disease: 6 - 9%	N/A	High
Lennie[54]	Yes, CHF	Stable patients only	100% CHF	N/A	High
O'Donnell[49]	Yes, prior CVD or high risk T2DM	Yes	Previous MI: 48% Prior stroke: 21%	Excluding events in the first year and excluding cancer events did not change results. Proportional hazards assumption not violated	Intermediate
Ekinci[64]	Yes, T2DM	Yes	Macrovascular disease: 43-49%	N/A	High

Supplementary Table 4a: Potential for Residual Confounding--Inadequate Adjustment

	Variables in model						
First Author	Calories or creatinine/weight?[a]	Blood Pressure (BP)	Missing key cardiovascular risk factors?	Missing key demographics?	Population	Strata	Inadequate adjustment
General Population with Diet Based Sodium Intake Measures							
Alderman, 1998[18]	Yes; and Na/kcal	Systolic blood pressure (SBP), hypertension (HTN)	Cholesterol, diabetes, smoking	Socio-economic status (SES)	General, US	None	Yes
He, 1999[17]	In model, sodium/kcal ratio is exposure variable	SBP, diuretic use	None	None	General, US	by BMI	No
He, 2002[52]	Yes	SBP	None	None	General, US	by BMI	No
Cohen, 2006[67]	Yes	SBP, BP treatment	None	None	General, US	None	No
Cohen, 2008[19]	Yes, in model unless exposure is already calorie adjusted	SBP, BP treatment	None	None	General, US	None	No
Yang[20]	Yes	No	Diabetes	None	General, US	None	No
Kagan[53]	No	No	Cholesterol, diabetes, smoking, body mass index (BMI)	SES	General, Hawaiian Japanese	None	Yes
Nagata[56]	Yes (in exposure variable)	Hypertension	Cholesterol	None	General, Japanese	By sex	No
Umesawa[61]	Yes (in exposure variable)	Hypertension	Cholesterol	None	General, Japanese	None	No
Takachi[58]	Yes (in exposure variable)	No	Diabetes, cholesterol	SES	General, Japanese	None	Yes
Gardener[63]	Yes	Hypertension	None	None	General, US	None	No
General Population with Urine Collection Based Sodium Intake Measures							
Tunstall-Pedoe[71]	No	No	Cholesterol, diabetes, smoking, BMI	SES	General, Scotland	By sex	Yes
Tuomilehto[60]	No	SBP	Diabetes	SES	General, Finland	None	Yes
Geleijnse[62]	Yes (creatinine)	Diuretics use	Cholesterol	None	General, Netherlands	None	No
Cook[66]	Yes (weight)	No	cholesterol	None	Prehypertensive not on HTN meds, non-diabetic, US	None	No
Stolarz-Skrzypek[47]	No	Anti-hypertensive drug use, SBP	None	None	General, Europe	None	Yes
Special Population with Diet Based Sodium Intake Measures							
Larsson[48]	Yes (in exposure variable)	SBP, DBP	None	SES	male smokers, Finland	None	No
Dong[65]	No (included in additional model)	No	Smoking	SES	Peritoneal dialysis, China	None	Yes
Arcand[68]	Yes	Beta-blockers, furosemide	Cholesterol, diabetes, smoking	race, SES	Heart failure, Canada	None	Yes
McCausland[55]	Yes (in Na/kcal analysis)	No	Cholesterol, smoking	SES	Hemodialysis, US	stratified by site, not shown	Yes
Special Population with Urine Collection Based Sodium Intake Measures							
Alderman, 1995[69]	No	SBP	BMI, diabetes	race, SES	Hypertension, US	Men only	Yes
Son[57]	No	No	Cholesterol, diabetes, smoking	SES	Heart failure, South Korea	None	Yes
Thomas[59]	No	SBP	Smoking	SES	Type 1 diabetes, Finland	None	Yes
Lennie[54]	No	No	Cholesterol, diabetes, smoking	race, SES	Heart failure, US	NYHA functional class	Yes
O'Donnell[49]	Yes (creatinine)	BP, change in SBP, hypertension, beta-blockers, diuretics, calcium antagonist	None	SES	Cardiovascular disease or type 2 diabetes, international	None	No
Ekinci[64]	No	SBP, ACE inhibitors	Cholesterol, smoking	SES, race	Type 2 diabetes, Australia	None	Yes

[a] Calories if Na assessment is diet based. Creatinine or weight if assessment is urine based.

Supplementary Table 4b: Potential for Residual Confounding--Imbalance Across Categories

Author	Age difference across Na intake categories	Race difference across Na intake categories	Sex difference across Na intake categories	Evidence of potential for residual confounding? (>5 yrs age difference or 20% race/sex)	Stratification done in analysis?	Imbalance Across Categories?
General Population Studies with Diet-Based Sodium Intake Measures						
Alderman, 1998[18]	Men: Q1: 56.9 yrs; Q4: 48.6 yrs Women: Q1: 49.8 yrs; Q4: 43.9 yrs	Men: Q1: 24% black; Q4: 8.8% black Women: Q1: 26% black; Q4: 11.5% black	Not given	Yes. Based on age	Stratified by age: < 65 v. >65. Results similar, but only significant in older group	No
He, 1999[17]	N: Q1: 46.2; Q4: 48.6 O: Q1: 50.0; Q4: 51.3	N: Q1: 82.3% white; Q4: 90% white O: Q1: 73.5% white; 82.4% white	N: Q1: 37.9; Q4: 42.0% male O: Q1: 35.9%; Q4: 32.7% male	No	N/a	No
He, 2002[52]	Can't assess	Can't assess	Can't assess	Can't assess	No	Can't assess
Cohen, 2006[67]	<2300: 49 yrs; >2300: 47 years	<2300: 11% black; >2300: 7%	<2300: 31% male; >2300: 60% male	Yes. Gender	Yes: no difference by sex.	No
Cohen, 2008[19]	Q1: 51 yrs; Q4: 44 yrs	Q1: 11% black; Q4: 8.4% black	Q1: 23.8% male; Q4: 68.1% male	Yes; age and gender	Yes, but no specific results reported for age or sex	Yes
Yang[20]	Can't assess	Can't assess	Can't assess	Can't assess	N/A	Can't assess
Kagan[53]	Can't assess	Can't assess	Can't assess	Can't assess	N/A	Can't assess
Nagata[56]	Men: T1:51 yrs; T3: 57.7 yrs Women: T1: 53.3; T3: 57.8 yrs	N/A	Stratified	Yes. Age.	Not for age.	Yes
Umesawa[61]	Quint 1: 55; Q5: 58	n/a	Q1: 55% male; Q5: 33%	Yes, for sex	No	Yes
Takachi[58]	Q1: 56.1 yrs; Q5: 57.9 yrs	N/A	Q1: 62% men; Q5: 32%	Yes, for sex	Yes: no difference by sex	No
Gardener[63]	<1500: 70; >4000: 68	<1500: 33% black; >4000: 21%	<1500: 21% men; >4000:49%	Yes, for sex.	No	Yes
General Population Studies with Urine Collection Based Sodium Intake Measures						
Tunstall-Pedoe[71]	Can't assess	Can't assess	Can't assess	Can't assess	N/A	Can't assess
Tuomilehto[60]	No difference	N/A	Can't assess (provides stratified analyses)	Can't assess for sex	Yes, by sex. Similar for all but all cause mortality and stroke. N in women too small to assess	Can't assess
Geleijnse[62]	Can't assess	Can't assess	Can't assess	Can't assess	N/A	Can't assess
Cook[66]	Can't assess	Can't assess	Can't assess	Can't assess	N/A	Can't assess
Stolarz-Skrzypek[47]	Women: T1: 42.5; T3: 39.2 Men: T1: 41.8; T3: 39.5	N/A	Can't assess	No	N/A -- note: tertiles are sex-specific.	No
Special Population Studies with Diet Based Sodium Intake Measures						
Larsson[48]	Q1: 57.3; Q5: 58.2	N/A	N/A	No	No	No
Dong[65]	T1: 63.1 T3: 54.2 yrs	N/A	T1: 20.8% male; T3: 63.7% male	Yes, for age and sex	No	Yes
Arcand[68]	T1: 62.1; T3: 57.4 yrs	Can't assess	T1: 66% male; T3: 90%	Yes, for sex	No	Yes
McCausland[55]	Can't assess	Can't assess	Can't assess	Can't assess	No	Can't assess
Special Population Studies with Urine Collection Based Sodium Intake Measures						
Alderman, 1995[69]	Men: Q1: 54; Q4: 50	Men: Q1: 41% white; Q4: 37%	Stratified by gender	No.	N/A	No
Son[57]	<3g: 64 yrs; >3g: 66	N/A	<3g: 75.6% male; >3 g: 68.7%	No	No	No
Thomas[59]	Q1: 38; Q4: 39	N/A	Q1: 32.6% male; Q4: 71.5%	Yes; for sex only	No	Yes
Lennie[54]	Can't assess	Can't assess	Can't assess	Can't assess	No	Can't assess
O'Donnell[49]	<2g: 67.61; >8g: 65.37	<2g : 63.7% white; >8: 73.2%	<2: 53.5% female; >8: 21%	Yes, for sex	Yes: by sex in univariate analyses. No difference	No
Ekinci[64]	T1: 67; T3: 61	Can't assess	T1: 42% male; T3: 70%	Yes, for age and sex	No	Yes

Appendix Table 5: Power and follow up

Author	# Events[a]	Power[b]	Loss to Follow Up
General Population Studies with Diet-Based Sodium Measures			
Alderman, 1998[18]	1790	0.99	0%
He, 1999[17]	N: 1080 O: 647	N: 0.94 O: 0.76	4%
He, 2002[52]	N: 413 O: 679	N: 0.58 O: 0.77	4%
Cohen, 2006[67]	541	0.71	0%
Cohen, 2008[19]	436	0.59	0%
Yang[20]	825	0.87	0%
Kagan[53]	238	0.34	Not given
Nagata[56]	M: 132 W: 137	M: 0.23 W: 0.24	5%
Umesawa[61]	1410	0.95	4%
Takachi[58]	2066	0.99	3.10%
Gardener[63]	615	0.74	Not given
General Population Studies with Urine Based Sodium Measures			
Tunstall-Pedoe[71]	M: 404 W: 177	M: 0.56 W: 0.29	0%
Tuomilehto[60]	128	0.21	0%
Geleijnse[62]	NA	NA	Not given
Cook[66]	193	0.31	24%
Stolarz-Skrzypek[47]	232	0.36	0% mortality; 22% other outcomes
Special Population studies with Diet-Based Sodium Measures			
Larsson[48]	2702	0.99	0%
Dong[65]	32	0.08	1.3% LTFU; 13% censored
Arcand[68]	73	0.15	0%
McCausland[55]	750	0.82	19% censored
Special Population Studies with Urine Based Sodium Measures			
Alderman, 1995[69]	117	0.15	4%
Son[57]	101	0.19	3%
Thomas[59]	217	0.34	0%
Lennie[54]	Class 1/2: 30 Class 3/4: 47	Class 1/2: 0.08 Class 3/4: 0.11	3%
O'Donnell[49]	4729	1	Not given
Ekinci[64]	75	0.15	3%

[a]Number of events represents highest number of CVD events. All cause mortality only used if it was the only outcome assessed

[b]Power to assess a 10% reduction in risk per 1 SD decrease in Na intake

[c]Case cohort

Table 6: Comparison of studies that use the same data with divergent results

Author, year	Population	Exclusions	Years of follow up	Variables in final model[b]	Sodium (Na) Intake variable	Strata	Results	Relevant sensitivity analyses
NHANES 1 Studies								
Alderman, 1998[18]	NHANES 1, 25-75	None	21	Age, sex, race, cardiovascular disease, hypertension, body mass index (BMI), systolic blood pressure (SBP), **table salt use,** total energy intake,	Na intake and Na/kcal ratio in the same model	None	Inverse significant for total mortality only	None
He, 1999[17 a]	NHANES 1, 25-74	Prior CV events and low salt diet for HTN	21	Age, sex, race, SBP, **cholesterol,** BMI, **diabetes, diuretic use, exercise, education, alcohol, current smoking**, total energy intake	Na/kcal (analyses also with Na intake)	Overweight v. Normalweight	Positive significant relationship for stroke, stroke mortality, CHD mortality, CVD mortality, and all cause mortality in **overweight population only**	None
NHANES 3 Studies								
Cohen, 2008[19]	NHANES 3, 30+	Prior CV disease, low salt diet, deaths within 6 months of FU, kcal intake <500, > 5000	8.7	Age, sex, race, education, **added table salt**, exercise, alcohol, current smoking, diabetes, **cancer, SBP,** cholesterol, **potassium, weight, hypertension treatmen**t, total energy intake	Na intake	None	Inverse, significant relationship for CVD mortality only	When Na/kcal or Kcal adjusted residuals used as intake variable, null results.
Yang, 2011[20]	NHANES 3, 20+	Prior CV disease and low salt diet	14.3	Age, sex, race education, **BMI**, smoking, alcohol, cholesterol, **HDL-C**, exercise, **family history of cardiovascular disease**, total energy intake	Usual Na intake, estimated using subset of population with a second day of dietary recall.	None	Positive, significant relationship for all cause mortality only	When Na intake from first day dietary recall used, null findings

[a] main exposure variable is sodium/kcal ratio, but since sodium intake is presented I have used that for comparability

[b] Bolded variables differ between the two studies

Intermap (INTERnational collaborative study of MAcronutrients, micronutrients and blood Pressure) Manual of Operations

Excerpts Related to 24-hour Urine Collection

III. URINE COLLECTION

24-HOUR COLLECTION - START

Two timed 24-hour urines are to be collected from each participant, 2 to 3 weeks apart.

The timing of the 24-hour urine collection starts immediately after the participant has voided at the clinical center. This casual sample is discarded.

1. Record name, ID, and split sample ID if assigned, onto the **Urine Collection Register** (see Appendix B).
2. Enter the date and time at the start of the 24-hour collection.
3. Also enter the time on Form G1F or G1R and check off other relevant questions on that form.
4. Obtain a set of 4-6 24-hour urine collection jars already prepared with preservative. (See Section VIII on the Clinic Visit.) Write the number of jars given in the space marked "Jars Given", on the Urine Collection Register. Obtain a carrying case. For women, add an approved collection aid.
5. Write the participant's name and add initials to complete the ID code on jar labels and affix one on each jar. Number the jars (e.g., from 1 to 5) as they are given to participant, writing the number on the jar label and entering the appropriate number of jars on the Urine Register (see Appendix B).
6. For 24-hour urine specimens to be used in the evaluation of daily electrolyte and protein intake, it is essential to ensure as far as possible that collections are complete for an accurately described time period. Therefore careful instruction of study participants is of

decisive importance.

7. Instruct the participant in the use of the jar and collection aid. While use of the aid is essential for women, it can be optional for men. Indicate that the jar should always be held during voiding (men) or pouring (when aid is used).

8. To start the collection, ask the participant to completely empty his or her bladder. Discard this sample.

9. All urine voided from that moment, including the remaining time spent at the clinic, is to be collected until the same time the following day.

10. Many people when having a bowel movement involuntarily also urinate. To avoid loss of this urine, explain to participants that when they feel the urge to have a bowel movement, they are to first pass urine into the collection jar, emptying the bladder completely.

11. Tell participant that when any jar is about 2/3 full, a new jar is to be started with the next voiding. This is to prevent overflow.

12. Give the participant the jars, collection aid, carrier, and Instructions sheet (see Appendix B) and ask to return to the clinic the same time the following day for supervised completion of the 24-hour collection. Ensure that time of return is noted in the clinic's reception diary and on the Instructions sheet.

Notes

1. If a participant is unable to empty his/her bladder, wait half an hour and ask the participant to try again. If the participant is still unable to void, measure the pulse and blood pressure, and then give the participant several glasses of water to drink. Proceed with the remainder of the clinic visit until the participant indicates that he or she is able to void. Then start the 24-hour collection in the usual way, discarding this sample.

   In making the first day visit appointment, inform the participant that he/she will be asked to void upon arrival at the clinic, and request that he/she try to avoid complete emptying of bladder right before coming to the clinic.

2. One-liter reusable plastic jars, with lids that screw on tightly to prevent leakage and which can be washed well between users, are to be ordered through the London or Chicago Coordinating Center. While 24-hour volume varies among individuals, each participant is to be provided with the likely maximum -- equivalent to a capacity of 4 or 5 liters. If experience in the individual populations, which may differ in output based on climate, usual intake, etc., shows that this number is too small or too large, adjustment is to be made.

3 The carrier appropriate for each population, and even within a population, can be expected to vary. Attache cases, camera bags, shopping bags with a firm bottom can be used. Local customs are to help determine the type of carrier. A key point is that the carrier is to help prevent the jars from tipping, with loss of some of the specimen, and with inconvenience to the participant. If possible, carriers are to have a foam rubber lining.

COMPLETING THE 24-HOUR COLLECTION

1. To ensure that the end of the 24-hour urine collection is correctly completed, the participant is to return to the clinic shortly before the 24 hours are over, and the final urine specimen is to be collected at that time. Ask the participant to empty his/her bladder completely at that time, into one of his/her jars.

2. Do not be concerned if the collection time varies slightly from 24 hours since this can be accounted for in the data analysis, as long as the actual time of completion is recorded by the staff member.

3. **Remember that the final sample of urine voided is to be included in the 24-hour collection.** Be sure to ask the Completeness Questions on Forms U2F or U2R and the special question on menstruation for women. Check that all equipment has been returned (urine collection jars, collection aid, and carrier) and that the collection jars are correctly labelled.

4. Enter the end time, and day of the week, as a check, on Form U2F or U2R. In addition, the end date and time, the number of urine collection jars returned, and the number of jars used in the 24-hour urine collection are to be recorded in the Urine Collection Register.

5 If the Completeness Questions indicate that a jar containing urine was not returned, and efforts to retrieve the urine specimen were not successful, or urine was voided other than into the jar, or more than a few drops were missing, e.g., through spillage, then this is to be considered an incomplete collection for data analysis. (If the amount lost is insignificant, e.g., from splashing, this is not considered incomplete.) Aliquots from

incomplete urines are also to be sent in the usual way. They can still supply some data possibly

relevant to our study, even if not part of the 24-hour data. As noted earlier, in this circumstance, the center is to either offer an option of a substitute pair of visits including urine collection, or recruit a supplementary participant in the same age-sex group to ensure that the center supplies 260 complete 24-hour collections for the first pair of visits and 260 complete 24-hour collections for the second pair of visits (repeat pair of visits). See the sampling instructions for how this person is to be selected for recruitment. Use the supplementary ID codes for this new person.

If the original participant wants a "second chance" at completing a 24-hour collection, data on the incomplete pair of visits are filed in a special folder and not entered into computer. For the new, substitute pair of visits, the same participant ID used in the original visits is used, as are the same numbers of forms (U2F or U2R, M and G forms, etc.).

REPEAT 24-HOUR URINE COLLECTION

1. All participants are to be asked to return for a second pair of visits (repeat pair of visits) 2 to 3 weeks after the first pair of visits, and they are to be asked to repeat the 24-hour urine collection at that time.
2. A random 10% of participants are to provide split samples; they will be the same individuals who provided split samples in the first pair of visits. (Note: these individuals are identified in the Log Book by split sample ID codes.)

3. The repeat appointment includes pulse, blood pressure, height and weight measurement, record of current medications, 24-hour dietary recalls (including alcohol and supplement information, where appropriate), and the second 24-hour urine collection. At the end of the first pair of visits, give the participant the repeat pair of visits instruction sheet, for attending the clinic at the appointed time. Remind the participant that at the second pair of visits (repeat pair of visits), collection of urine is again to be started at the clinic and completed at the clinic 24 hours later.

THE URINE COLLECTION REGISTER

1. The local center laboratory is to keep a record of all specimens received, number of jars distributed, number returned, and number of jars used in 24-hour urine collections, to help check on completeness. In addition, dates of refrigeration, freezing, and shipment of aliquots from the clinic, and visit number are to be recorded. All this information is to be recorded on the Urine Collection Register, a copy of which is in Appendix B. A copy of this form (with names removed) is to be sent monthly to the London Coordinating Center.

2. The procedure is as follows:

   a. At the start of the 24-hour urine collection, when the collection jars are labelled, numbered and given out, the participant's name, ID, and split sample ID, if assigned, are to be entered into the Urine Collection Register.

   b. Enter the start date and time of the 24-hour urine collection. This information is to be on Form G1F or G1R, depending on whether the visit is a first or repeat pair of visits, and also in the Register.

   c. Write down the number of jars that were given for the 24-hour urine collection. This is generally to be 4 or 5.

3. Twenty-four hours later, at the completion of the urine collection:

   a. Record the end date and time of the urine collection. This information is to be both on Form U2F or U2R and on the Urine Collection Register. Also, enter

number of jars returned and number of jars used during the 24-hour collection. If any jars are missing, ascertain whether these jars contained any of the urine

passed during the last 24 hours. If a jar or jars was used but not returned, make every effort to retrieve the missing urine that day (for example, if it was left at home but is still intact). If this is not possible, make a note on Form U2F or U2R. (In any case, try to retrieve the missing jars since otherwise there will not be enough jars to meet your requirements for the study.) A supplementary participant in the same age-sex group is to be randomly selected.

b. Remainder of the Urine Collection Register is filled out as the specimens are prepared in the laboratory. The items to be entered include:

checking the split column if blinded split samples are being sent to the laboratory;

date of refrigeration, freezing, shipping.

c. The Urine Collection Register is a working form for the center. Copies of completed pages (dated) are to be sent regularly to London (<u>without</u> participant name) when participant data are shipped.

MEASURING URINE VOLUME

The 3 values that are needed to assess 24-hour electrolyte and urea nitrogen output are:

concentration (reported, for example, as milliequivalents per liter), determined by the Central Laboratory

volume (number of milliliters)

time (number of hours and minutes)

We have discussed the importance of accurate timing. The next critical step is correct measurement of the urine volume.

1. Since we are all using standard collection jars supplied centrally (1-liter capacity and uniform height), height of the urine in the jars (in centimeters) is measured and later is converted into volume (ml) by computer in London. A computer conversion program has been developed for this purpose. This eliminates need to measure by pouring.

2. Height of urine in each jar is to be determined using the special platform with attached vertical scale (provided centrally). Record the value onto Form U2F or U2R, in centimeters to the nearest 0.1 cm. Each jar is to be read and recorded separately. Total height of the urine for the entire specimen and hence the volume are to be calculated by computer in London. Note that each line on the measuring scale represents 0 1 cm.

   Each jar is to be numbered on the jar label when it is given out to the participant. Record height of urine in each jar in the appropriate (numbered) space on Form U2F or U2R, as soon as it is measured, and set the jar aside. This is to reduce the risk of measuring the same jar twice, or missing a jar. For added security, note down on Form U2F or U2R the number of jars containing urine (the same number recorded in the "Jars Used" column of the Urine Collection Register).

   If there is foam at the top of the urine column, let jar rest a while. If it remains, measure up to the foam.

   There is room on Forms U2F and U2R to record height of urine in each of 6 1-liter jars, although in most cases the number of jars used will be less than 6. Put zeroes in the space provided, for jars not used.

3. In reading the line on the scale nearest the top of the urine, the collection jar is to be at eye level. This means either that the observer be seated at a table sufficiently high to

permit this, or that there be an eye-level shelf on which the measuring platform is placed. Jars are to be perfectly upright when read. If the top of urine falls halfway between two 0.1 cm lines, record the higher value.

4. The next step after carefully recording height of urine in the collection jars is preparing the sample aliquots.

PREPARING TO TAKE ALIQUOTS

1. Remember to take aliquots from 24-hour urine collection of one participant at a time, to avoid confusing collections from different participants.

2. To prepare to take aliquots from the 24-hour collection:

After the height of urine in each jar has been recorded, pour all the urine of the individual into a large bucket and thoroughly mix all the urine from the 24-hour collection of the individual participant. Since electrolyte output may differ at different times of the day, this mixing is very important. The mixing should not be done too rapidly or vigorously, in order to avoid spill and frothing.

Before describing how the aliquot tubes are to be filled, etc., it is necessary to describe the labelling procedure.

LABELLING

Chicago is to supply each center with the necessary number of sticky labels (see Appendix B for sample of labels).

1. We have already mentioned the "JAR LABEL" to be placed on the collection jars given to the participant. They have space for writing participant's name and for completing the participant's ID code by adding initials, and say "JAR LABEL" on them.

2. We also mentioned the FILE LABEL for the participant's file folder, with participant's ID code (add initials) and split sample ID, if assigned

3 All labels for the urine collection on the first set of visits have the ID number already printed on them, and also indicate how they are to be used. The participant's initials (first name and surname only) need to be added to make up the ID code.

IDs are also to be printed in advance on labels for the urine collection for the second pair of visits (repeat visits) and also for the split samples.

4. Each label has, as the last digit, a number indicating its purpose.

Label Number 1 = 24-hour urine aliquot for the Central Laboratory for current analyses.

Label Number 2 = 24-hour urine aliquot to freeze and store locally (this is a backup against possible loss in transit)

Label Number 3 = 24-hour aliquot for Central Laboratory (stored at Central Laboratory at -25^{0}C, as back-up for current analyses)

Labels Number 4 and 5 = 24-hour aliquot for Central Laboratory (stored at Central Laboratory at -80^{0}C for possible future analyses)

5. The identifying information on the labels is laid out in the following way:

Urine specimen (URI)

Participant ID or Split-sample ID numbers

Space for initials

First pair of visits (F) or second pair of visits (repeat visit) (R)

Label number

You need to fill in the initials that are part of that participant's code.

A 24-hour aliquot label for local storage for the first set of visits of a participant looks like this:

URI 66063 _ _ F 2 (the "URI" is an abbreviation for urine)

Again, fill in the initials (first initial of first name and of last name).

6. If this participant has been assigned a split sample ID, then another complete set of pre-printed labels with that ID has been prepared for him/her centrally.

As an example, suppose the participant used in the earlier example (66063 DK) also has a split sample ID code. Then, the label for the split sample of the 24-hour aliquot for local storage for this visit looks just like the above label, but substitutes the split sample ID number for the original one:

URI 66032 _ _ F 2

(Fill in the artificial split sample initials as printed in the Log Book to complete the split sample ID code. If these had been printed in advance by Chicago, then the Central Laboratory could identify the sample as a split sample.)

7. The entry (in letters) on the left side of the label is:
URI (for each 24-hour urine aliquot)

8. The center of the label is for the participant ID or the split sample ID, with numbers printed in advance. Initials are always added locally to complete the participant ID The first two digits of this ID number are always the code for your center

9. The right side of the label is for indicating two other items:
Visit Number: either F (first pair of visits) or R (second pair of visits -- repeat visit)
Label Number: 1 to 5 (to identify type of specimen and whether to ship or store -- see above)

10. Chicago is providing extra blank labels, for spoiled labels.

11. Chicago is also providing special labels for the 'Dry Run' (pilot field study) marked with a temporary center number.

12. When writing on labels, use the indelible waterproof marking pen provided centrally.

TAKING ALIQUOTS

1. Prepare one set of urine aliquots at a time to avoid confusing urine collections from different participants.

2. Each center is to receive aliquot tubes with push tops, supplied centrally. Their capacity is 9 milliliters (approximately 7 cm in height), but do not fill them to the top, since they can then break on freezing. Leave a gap of about 2 cm at the top of each tube. Make sure tops are pushed firmly down into the tubes (i.e., until you hear a click).

3. Complete the correct labels (those marked "URI" -- meaning for the 24-hour urine collection). Apply them to the tubes and cover the label with transparent tape wrapped around the tube.

4. Next, fill 5 aliquot tubes from the bucket containing the mixed 24-hour specimen. Use a pourer or pipette. Again, be sure that all jars of the collection have been measured, and then mixed together. (If there is a split sample ID, then 10 tubes are required -- 5 with the real ID and 5 with the split ID number.) Fill tubes as above, leaving 2 cm unfilled and snap the tops in securely (until you hear a click).

STORING THE URINE ALIQUOTS

1. Although use of preservative to some extent protects the urine specimen against bacterial growth, there is nevertheless a deterioration in creatinine which becomes apparent within 24-48 hours at a temperature of +20° C. All jars should be refrigerated until aliquots are

prepared and aliquots are to be taken within one week of receipt. All aliquots must be refrigerated at 4°C

or less within 24 hours and frozen at -20°C within seven days.

2. On-site refrigerators (or freezers) need back-up electrical supply, for example, car batteries, to take over in case of breakdown.

3 If there is no freezing facility at the local clinic, urine aliquots will require local transportation for freezing elsewhere. They must be transported in refrigeration boxes. Glycerine-based cooling elements (supplied if needed from the Central Laboratory for placement in the refrigeration boxes) are first to be frozen at -20°C for at least 12 hours at the location where the specimens are to be stored prior to air shipment to the Central Laboratory. A maximum of six hours each way can then be allowed for transport between the clinic and local storage (with freezer). It is essential that local centers are able to comply with the above requirements – if more than twelve hours total elapse, the cooling elements thaw.

4. Refrigeration and freezing dates are to be entered in the URINE COLLECTION REGISTER.

SHIPPING URINE ALIQUOTS

1. The Central Laboratory for all chemical analyses of urine is at the St. Rafael University Hospital, Capucijnenvoer 33, B-3000 Leuven, Belgium. Shipment of urine aliquots is to be by air freight.

2. Urine aliquots must be shipped frozen (-20°C) on dry ice.

3. Shipment of aliquots is to be as follows:

The first shipment is of the dry run (test run) specimens.

The next shipment is to be after two weeks of data collection, or after 25 urine collections have been made, whichever is the sooner; other shipments are to be made every 3 months during data collection.

PROCEDURE FOR SHIPPING URINE ALIQUOTS

1. Make contact with the nearest and most reliable courier service that can deliver to the Laboratory.
2. Notify them that samples must be kept at -20°C, both in the airport of departure while awaiting transfer to the aircraft and in the aircraft itself.
3. Arrangements are to be made at least two weeks before date of shipment. Local Investigators are to make first contact with the courier before the dry run begins, to determine local regulations and procedures.
4. Two weeks before shipment, send a fax, e-mail message, or telex to the Central Laboratory and similarly to the London Coordinating Center, to alert them to the coming shipment.

   The fax, E-mail, or telex (see below for these numbers) is to contain as much of the following information as is available at that time, with the rest supplied prior to shipment.

INTERMAP CENTRAL LABORATORY

C/O ST. RAFAEL UNIVERSITY HOSPITAL - LEUVEN - BELGIUM

ATTN: PROF. HUGO KESTELOOT

CENTER: .....

AIRPORT OF DEPARTURE: .....

DATE OF SHIPMENT: .....

LOCAL HOUR OF SHIPMENT: .....

NUMBER OF PACKAGES: .....

AIR FREIGHT OR COURIER NUMBER: .....

FLIGHT NUMBER(S) IF APPROPRIATE: .....

DATE AND TIME OF SCHEDULED ARRIVAL IN LEUVEN: .....

LOCAL INVESTIGATOR: .....

For the London Coordinating Center the first three lines have to be replaced by:

INTERMAP COORDINATING Center (see below for address, phone, fax and e-mail numbers)

ATTN: DR. DEBORAH CHEE

5. If no Fax, e-mail facility, or telex machine is available locally, it is critically important that the above information be received by the Central Laboratory before shipment, so that the shipment can be anticipated. In these circumstances, two weeks before shipment, an international telegram is to be sent or a call made to London and London is to advise the Laboratory.

6. Fax, e-mail or telex messages are also to be sent (preferably from the airport) on the day of shipment.

7. Enter shipping date from the clinic in the center's Urine Collection Register.

8. Packing the urine aliquots for shipment

Be sure the packing box is large enough to permit a sufficient quantity of dry ice to be used, to guarantee that specimens arrive frozen. Check with courier on any special regulations.

9 Ensure that the courier is aware that specimens are on dry ice and are to be shipped at -20°C and that information is passed on to air carrier.

10. The following labels are to be affixed to the outside of the box:

A large label with the preprinted address of the Central Laboratory;

A return label with the address of the local Investigator;

Special labels indicating the nature of the content of the refrigeration box:

HANDLE WITH CARE

DEGRADABLE GOODS TO BE KEPT AT -20°C

DRY ICE

NO COMMERCIAL VALUE

All these labels are provided centrally.

Note:

1. Back-up urine aliquots are to be kept locally at -20°C until clearance has been received from the London Coordinating Center.

2. Dry ice is necessary for shipments since the integrity of specimens on wet ice cannot be guaranteed for more than a few hours and urine specimens may thaw out. Use of dry ice needs to be cleared with the courier. Packing procedures must be in accordance with instructions agreed with the airline and/or courier company.

COMMUNICATION PROCEDURE

On receipt of urine aliquots in Leuven, the Central Laboratory is to inform the London Coordinating Center by fax of the number of aliquots received, the condition in which they were received, and of any discrepancy noted. For example, if one of the four requested urine aliquots (aliquot numbers 1, 3, 4, 5) is missing for a particular participant, or if ID code is incomplete, or if samples are thawed, this is to be reported immediately to London, so that the London Coordinating Center can rapidly inform the local center of problems and of actions to be taken.