Agreement Between 24-Hour Salt Ingestion and Sodium Excretion in a Controlled Environment

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Abstract—Accurately collected 24-hour urine collections are presumed to be valid for estimating salt intake in individuals. We performed 2 independent ultralong-term salt balance studies lasting 105 (4 men) and 205 (6 men) days in 10 men simulating a flight to Mars. We controlled dietary intake of all constituents for months at salt intakes of 12, 9, and 6 g/d and collected all urine. The subjects' daily menus consisted of 27 279 individual servings, of which 83.0% were completely consumed, 16.5% completely rejected, and 0.5% incompletely consumed. Urinary recovery of dietary salt was 92% of recorded intake, indicating long-term steady-state sodium balance in both studies. Even at fixed salt intake, 24-hour urine collection for sodium excretion (UNaV) showed infradian rhythmicity. We defined a ±25 mmol deviation from the average difference between recorded sodium intake and UNaV as the prediction interval to accurately classify a 3-g difference in salt intake. Because of the biological variability in UNaV, only every other daily urine sample correctly classified a 3-g difference in salt intake (49%). By increasing the observations to 3 consecutive 24-hour collections and sodium intakes, classification accuracy improved to 75%. Collecting seven 24-hour urines and sodium intake samples improved classification accuracy to 92%. We conclude that single 24-hour urine collections at intakes ranging from 6 to 12 g salt per day were not suitable to detect a 3-g difference in individual salt intake. Repeated measurements of 24-hour UNaV improve precision. This knowledge could be relevant to patient care and the conduct of intervention trials. (***Hypertension***. **2015;66:850-857. DOI: 10.1161/HYPERTENSIONAHA.115.05851.)**

● **Online Data Supplement**

Key Words: hypertension ■ salt ■ sodium ■ sodium, dietary ■ urine specimen collection

Opinion leaders advocate a reduced salt intake diet to lower blood pressure in the general population.[1,2] The World Health Organization (WHO) currently recommends consuming <5 g of salt daily.[3] Given prevailing food consumption patterns and the current food supply, implementing the WHO guidelines will be an enormous challenge for global public health. The decision making was based on trials that explored the relationship between sodium and cardiovascular disease.[4–6] The analytic gold standard to determine dietary salt intake is a 24-hour urine collection for sodium excretion (UNaV).[7,8] A systematic review of 31 sodium–cardiovascular disease cohort studies indicated that only 2 studies used repetitive 24-hour urine collections to assess salt intake.[9] The 2013 Institute of Medicine report found the existing studies to be highly variable in methodological quality, particular in assessing salt intake.[2] The 2012 World Health Organization report identified the quality of the evidence on the relationship between sodium intake and cardiovascular disease as very low.[10] Because public policy apparently is based on such data, a critical evaluation of a widely accepted technique to estimate salt intake in humans could be particularly important. We recently conducted a long-term salt balance study in men simulating a

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flight to Mars. We varied salt intake between 12 and 6 g/d, all food intake was precisely determined, and all urine made was collected in 2 studies of 105 and 205 days duration, respectively.[11] The intake of any-and-all other dietary constituents was maintained constant. We observed astonishing variability in UNaV and hypothesized that 24-hour samples are not reliable estimates of salt intake across the range tested. We find that a single, accurately collected, 24-hour urine sample was not suitable for determining a 3-g difference in the subjects' daily salt intake. Our findings suggest that estimating individual salt intake from urine collections could be less precise than supposed.

Methods

We performed 2 long-term studies in the framework of a simulated flight to Mars, conducted in Moscow, Russia, from 2009 to 2011. We have presented a detailed description of the experimental approach previously.[11] The study was conducted at the Institute for Biomedical Problems in Moscow. Several ethical review boards approved the studies including the internal review board equivalent of the Russian Federation. Written informed consent was obtained from all participants, and all studies were performed as outlined in the Declaration of Helsinki.

Study Design and Oversight

Ten healthy male volunteers lived for 105 days (Mars105) and 205 days (Mars520) in an enclosed habitat consisting of hermetically sealed interconnecting modules. Microgravity and space radiation were not simulated. Selection criteria for the volunteers for the simulated mission to Mars were equal to that of real cosmonauts. No subject had any known medical condition and none ingested medications for any reasons. Environmental factors were maintained constant and enabled a metabolic ward setting for this experiment. Temperature and relative degree of humidity were maintained between 18°C and 25°C and between 30% and 85%, respectively. During the study, subjects had free access to water, although fruit juices were limited. The crews lived and worked like cosmonauts on the international space station. Daily calorie intake was between 2500 and 2800 kcal per day, satisfying the energy requirements for a light activity lifestyle that characterizes daily life conditions in industrial societies.[12] Movement activity was continuously measured with wrist actigraphy in the 6 subjects participating in the Mars520 study.[13]

Nutritional intervention took place during the complete Mars105 study and the first 205 days of the simulated flight to Mars during the Mars520 study. The design of the study featured decremental decreases in salt intake from 12 to 9 g to 6 g salt per day (Figure 1A). There was a brief period of reexposure to 12 g between 9 and 6 g salt per day in Mars105, for reasons not relevant to this study. Because of the longer duration of the Mars520 study, the intake phases were 12, 9, 6 g, and re-exposure to 12 g salt per day. Furthermore, the Mars520 subjects rejected some offered salty servings, resulting in 0.2 to 0.8 g per day lower recorded salt intake levels during the Mars520 balance study. Prescribed average salt intake, energy intake, carbohydrate intake, fat intake, protein intake, and fiber intake were maintained constant (Tables 1 and 2), tightest in the 105-day study and slightly less stringent in the 205-day study. This strict constancy in all nutrients, except for dietary salt intake, allowed isolated assessment of salt consumption effects on sodium excretion.[11]

We could not ask the subjects to eat the same breakfast, lunch, and dinner for weeks on end. Thus, we designed menus from varied processed foods that maintained all constituents as constant as possible, modifying only the salt intake. The cationic content of these foods has been determined by chemical analysis as required by regulatory food agencies. Each subject had an individual daily menu plan, which listed all food products he was to consume on a particular day. Each subject was asked to adhere to the menu plan as strictly as possible and consume every listed item. Each subject was also asked to document directly onto the menu plan (daily diary) in percentage, if he failed to eat the contents of any food items completely. The nutritionist afterward made necessary adjustments to the amount of ingested nutrients of the subject on that day, resulting in precise information on the long-term prescribed, and the day-to-day recorded salt intake. In total, the day-to-day recorded food products contained 14.8 kg salt,

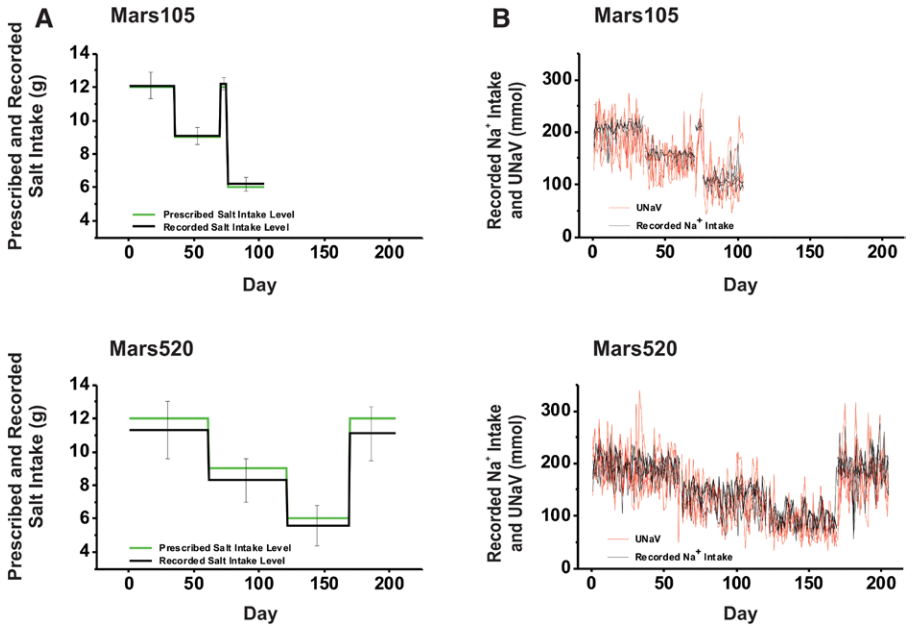

Figure 1. Study conduct and variable sodium excretion. **A**, Shown are the prescribed (green) and mean recorded (black) salt intakes (12, 9, and 6 g/d) at each phase in 105 days (Mars105) and 205 days (Mars520). In Mars520, the prescribed salt intake was higher than the recorded salt intake because of subject preference. The brief reexposure to 12 g/d in Mars105 was done for reasons outside our study. In Mars520, we performed a lengthy reexposure to 12 g/d. **B**, Recorded sodium intake (mmol/d, black) and 24-hour urine collection for sodium excretion (UNaV; red) of all subjects during the Mars105 and the Mars520 study.

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Table 1. Anthropometric Data and Nutrient Intake During the Mars105 Study (Mean±SD)

	Mars105 Study		
Salt Intake Level	12 g for 35 d	9 g for 35 d	6 g for 29 d
Age, y	34.3±5.2	34.3±5.2	34.8±4.3
Height, cm	178.3±4.5	178.3±4.5	178.3±4.5
Body weight, kg	77.7±9.8	77.0±8.2	76.2±7.2
Energy intake, kcal/d	2775.0±250.5	2818.2±179.6	2711.9±311.4
Carbohydrate intake, g/d	371.8±46.4	387.4±36.6	384.3±50.5
Fat intake, g/d	89.7±12.2	87.6±8.9	80.3±14.2
Protein intake, g/d	103.5±11.9	101.4±10.9	93.4±16.0
Fiber intake, g/d	29.7±7.0	27.5±5.8	27.7±6.6
Salt consumed, kg	1.688	1.279	0.718
Salt excreted in urine, kg	1.529 (91%)	1.188 (93%)	0.743 (104%)

Total salt consumption was calculated from protocolled food intake. The total salt excretion in urine is also expressed as percent of intake.

of which we found 13.7 kg in the subjects' urine (Tables 1 and 2). Recovery of dietary salt in urine thus was 92% of intake, indicating long-term steady-state sodium balance in both studies. Subjects were allowed to drink water ad libitum, measured fluid intake gravimetrically, and recorded the intake amounts directly onto the menu plans. The subjects collected all their urine for a 24-hour period every day and determined the urine volume gravimetrically. Urinary creatinine excretion was constant at all salt intakes (Figure S1 in the online-only Data Supplement). Less than 0.1% of our 1646 urine samples showed creatinine excretion below 0.8 g/d or above 2.4 g/d. We therefore used all samples for analysis.

Biochemical Analyses

The food industry supplying the food chemically analyzed electrolyte content in their products. We analyzed urine sodium content with flame photometry. Creatinine was measured by an automated technique. Daily renal sodium excretion as 24-hour UNaV was calculated by multiplication of urine sodium concentration and urine volume.

Statistical Analyses

To analyze the predictive value of a single 24-hour urine sample to accurately estimate real salt intake, we compared true salt intake with measured 24-hour sodium excretion in the urine. Because current computerized models often calculate the projected effect of a 3-g reduction in salt intake on cardiovascular outcome, we tested the accuracy of UNaV to correctly estimate real salt intake within a 3-g (50 mmol) range. Accuracy of each individual UNaV for correct assessment of daily salt intake was performed by definition of true positives of salt intake. We investigated the difference between recorded sodium intake and UNaV with Bland–Altman plots (Figure S2). We considered a ±25 mmol (corresponding to ±1.5 g salt) deviation of the mean difference between the recorded sodium intake and renal sodium excretion as true positive urine sample (correct prediction of salt intake). UNaV samples, which were outside this range, were considered as true negative (misclassification of salt intake). To test the effect of salt intake on UNaV measures, we conducted multilevel modeling using linear mixed models. We tested a random intercept versus a random intercept–slope model and selected the best-fit model. A P value <0.05 was considered statistically significant. Data analysis was performed with IBM/SPSS software (Version 20.0, IBM Corporation, Armonk) and R (Version 3.1.1 R Foundation for Statistical Computing, Vienna, Austria) using the packages lme4 and nlme.

Results

Anthropometric data and nutrient intake during the 2 Mars simulation studies are given (Tables 1 and 2). The complete menus during the Mars105 and the Mars520 study consisted of 27 279 individual servings, of which 22 635 (83.0%) were completely consumed and 4494 (16.5%) were completely rejected (Table S1). The number of incompletely consumed servings was 150 (0.5%). The original time series of recorded sodium intake and UNaV from all 4 subjects participating in the Mars105 and the Mars520 balance studies is given in Figure 1B. The daily UNaV fluctuated around the daily salt intake. In the 205-day study, we were not able to clamp intake to the same rigor as in the 105-day study, resulting in higher variability in recorded daily salt intake.

The relationship between recorded sodium intake and UNaV was direct (y=0.80x+20.0; Pearson's r=0.69; Figure 2A). However, the daily 24-hour UNaV in any individual was highly variable at each intake level (Figure 2B). Linear mixed model analysis confirmed that all individual subjects uniformly exhibited a direct relationship between

Table 2. Anthropometric Data and Nutrient Intake During the Mars520 Study (Mean±SD)

	Mars520 Study			
Salt Intake Level	12 g for 61 d	9 g for 60 d	6 g for 60 d	12 g for 36 d
Age, y	31.5±5.0	32.0±5.1	32.0±5.1	32.2±5.0
Height, cm	176.5±3.3	176.5±3.3	176.5±3.3	176.5±3.3
Body weight, kg	84.2±7.5	83.2±6.5	81.6±6.6	81.4±6.7
Energy intake, kcal/d	2649±185	2572±232	2472±221	2472±322
Carbohydrate intake, g/d	337.8±37.0	314.8±36.2	290.3±36.5	315.4±46.8
Fat intake, g/d	96.1±14.3	100.8±14.9	100.9±15.7	89.7±19.3
Protein intake, g/d	93.8±14.7	93.4±13.5	91.3±10.3	88.1±16.7
Fiber intake, g/d	34.5±8.0	37.0±6.7	36.0±7.4	32.6±7.8
Salt consumed, kg	4.138	2.977	1.624	2.389
Salt excreted in urine, kg	3.973 (96%)	2.637 (89%)	1.408 (87%)	2.208 (92%)

Total salt consumption was calculated from protocolled food intake. The total salt excretion in urine is also expressed as percent of intake.

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sodium intake and UNaV (y=0.78x+23.6; Pearson's r=0.69; 95% confidence interval, 0.661–0.712). Average UNaV thus provided the expected valid estimate of mean salt intake and recorded sodium intake in all subjects, albeit at high day-to-day variability of UNaV.

We therefore next tested the use of 24-hour UNaV for predicting daily salt intake in our subjects (Figure 3). Bland–Altman plots visualize the agreement between 2 signals. We considered a ±25 mmol (corresponding to approximately ±1.5 g salt) deviation in UNaV from the mean difference between recorded sodium intake and UNaV as correct prediction to separate 3-g differences in salt intake (prediction interval). We quantified the number of observations within the 3-g salt prediction interval after transfer of our original recorded sodium intake and UNaV into Bland–Altman plots. The mean difference between 1646 single 24-hour UNaV samples and their corresponding recorded daily sodium intake was 12±39 mmol/d (salt: 0.7±2.0 g/d), indicating normal extrarenal sodium loss and steady-state sodium balance at all salt intake levels (Figure 3A). Every other single UNaV sample was not within the 3-g prediction interval and misclassified daily recorded sodium intake (Table 3). We next tested the effect of repetitive collections on the predictive value of UNaV. When we tested the agreement between 3 consecutive 24-hour sodium intake records with 3 consecutive UNaV samples, 75% of the UNaV sodium samples detected the 3-g range in salt intake (Figure 3B). Combining 7 recordings of sodium intake with 7 UNaV collections further increased the agreement between sodium intake and excretion, and 8% of the combined intake and excretion samples misclassified the 3-g range in salt intake (Figure 3C).

Under daily conditions, studies on prescribed salt intake usually do not provide with daily information on protocolled sodium intake. To investigate this situation, we next tested the agreement between average prescribed salt intake and UNaV. We defined a ±25 mmol deviation from the average prescribed salt intake level as acceptable agreement between prescribed salt intake and UNaV. This definition of the 3-g prediction interval neglects the difference between intake and excretion because of extrarenal sodium loss (12±39 mmol/d, Figure 3), and the existing difference between prescribed and daily recorded salt intake (8±22 mmol/d, Figure 1A), which results in an analytic systemic error that is almost 50% of the range of the selected prediction interval (±25 mmol). Comparable with Bland–Altman plot analysis, only every other single UNaV collection was within the predicted 3-g range of salt intake (Table 4). However, the systematic error of not involving extrarenal sodium loss and the difference between actual and recorded sodium intake into the equation significantly reduced the predictive value of repetitive UNaV measurements, because many UNaV values ranged below the defined prediction interval. Therefore, neither 3 consecutive nor 7 consecutive UNaV samples improved the predictive value of UNaV to accurately detect a 3-g range in salt intake beyond the coin-toss level when extrarenal sodium losses and the existing difference between prescribed and recorded salt intake were neglected (Table 4).

Discussion

The major finding of our long-term balance study is that steady state between sodium intake and excretion in contrast to traditional opinion,[2] is not achieved within several days, but rather that weeks or even months are required before output is similar to intake. We reported earlier that our long-term balance studies allowed identification of weekly and monthly rhythms of body sodium accumulation and release by spontaneous, rhythmical variability in aldosterone, cortisol, and cortisone that occurred independent of salt intake.[11] This major endogenous biological confounding variable had clinical implications because every second single 24-hour UNaV failed to

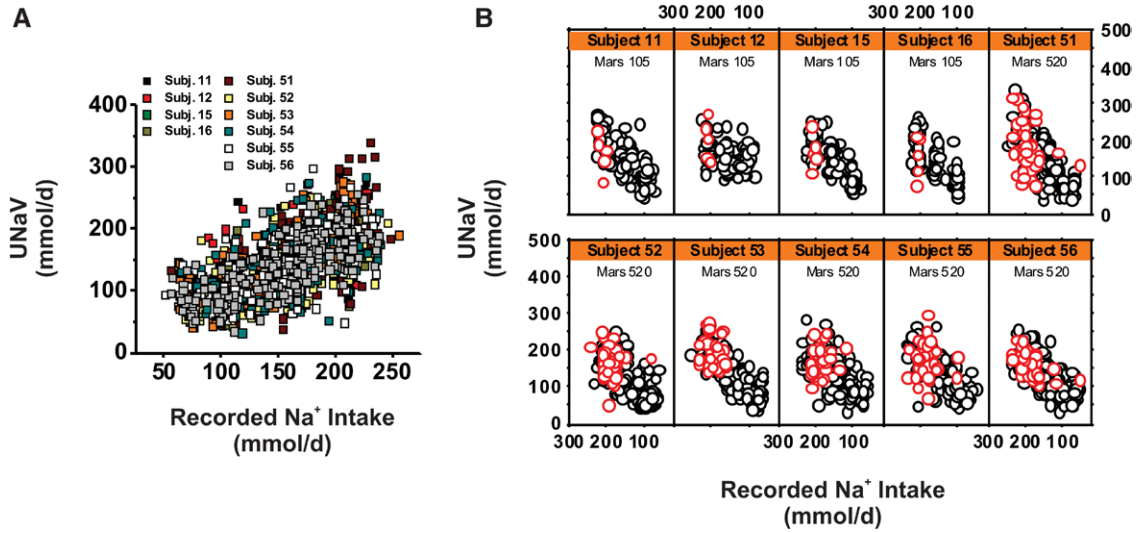

Figure 2. Relationship between recorded sodium intake and 24-hour urine collection for sodium excretion (UNaV; both mmol/d). **A**, Recorded daily sodium intake is plotted against 24-hour UNaV. **B**, The same relationship is shown for each individual subject. Red symbols are reexposure to 12 g/d salt intake. Mars105 subjects are numbered 11, 12, 15, and 16. Mars520 subjects are numbered 51 to 56.

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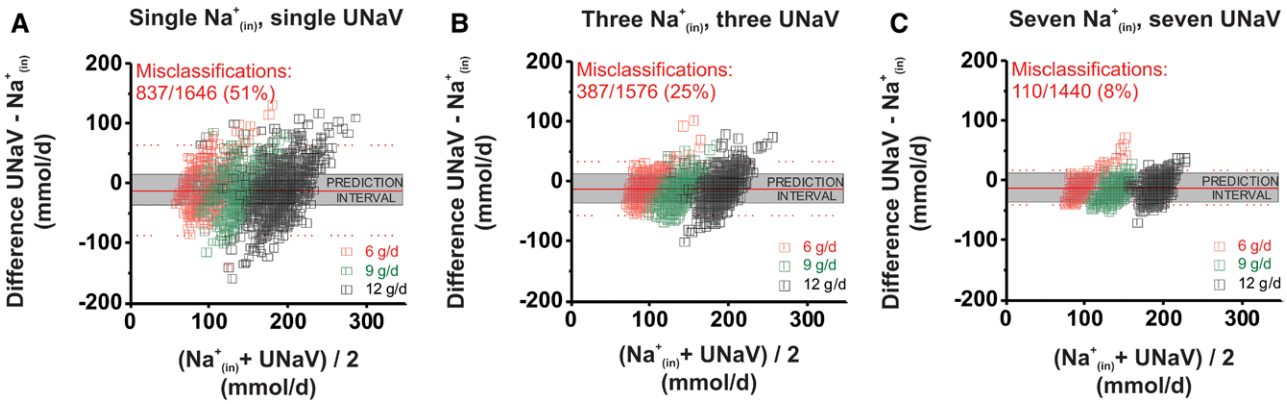

Figure 3. Analysis of agreement between sodium intake and excretion in Bland–Altman plots. **A**, Bland–Altman plot to test the agreement between single recorded 24-hour sodium intakes and single 24-hour urine collection for sodium excretion (UNaV). The prediction interval to accurately predict sodium intake by UNaV is defined as ±25 mmol/d of the mean difference between sodium intake and UNaV. **B**, Analysis of agreement between 3 consecutively recorded sodium intakes and 3 UNaV collections. Multiple collections reduce the variability and thereby improve the predictive value of UNaV. **C**, Seven consecutive collections reduce the number of misclassifications of UNaV to <10%. Red solid line indicates regression line; and red dotted line, upper and lower confidence level.

detect a 3-g difference in sodium intake. Multiple collections would improve precision.

Luft et al[14] made similar observations to those reported here in humans living under daily-life conditions. Their subjects varied their salt intake daily around a given mean intake, as is likely the case in real life, whereas in this study salt intake was more rigorously fixed. They found that 9 collections were optimal to predict salt intake, and that nocturnal (first-morning-voided urines) collections were of no value. In line with these findings, we show that collection of 3 consecutive 24-hour UNaV samples and actual sodium intake reduced the number of misclassifications to 25%, and a collection of 7 samples to 8%.

This obvious difficulty in correctly estimating salt intake even with repetitive measurements in normal humans under rigorously controlled environmental conditions illustrates the problem in categorizing actual salt intake in individuals. Because 24-hour UNaV is difficult to collect in free-living persons, the Kawasaki formula has been introduced to substitute for 24-hour collections.[15] Such studies have identified a U-shaped relationship between salt intake and cardiovascular risk.[16,17] Mente et al[18] have validated and compared the Kawasaki and other formulae to estimate 24-hour UNaV. They found an acceptable intraclass correlation coefficient between estimated and measured sodium excretion with the Kawasaki

Table 3. Agreement Between Recorded Sodium Intake and UNaV

Salt Intake Level	No. of Observations, n	Prediction Interval, mmol	UNaV Within Prediction Interval, n (%)	UNaV Outside Prediction Interval, n
Single recorded Na^+ intakes and UNaV collection				
12 g/d	742	−37.9 to 12.1	332 (45)	410 (a: 203, b: 207 55%)
9 g/d	500	−39.9 to 10.1	255 (51)	245 (a: 120, b: 125, 49%)
6 g/d	404	−33.1 to 16.9	222 (55)	182 (a: 84, b: 98, 45%)
All levels	1646	…	809 (49)	837 (51%)
Three recorded Na^+ intakes and UNaV collections				
12 g/d	712	−36.7 to 13.3	510 (72)	202 (a: 103, b: 99, 28%)
9 g/d	480	−40.2 to 9.8	373 (78)	107 (a: 48, b: 59, 22%)
6 g/d	384	−33.4 to 16.6	306 (80)	78 (a: 44, b: 34, 20%)
All levels	1576	…	1189 (75)	387 (25%)
Seven recorded Na^+ intakes and UNaV collections				
12 g/d	656	−36.0 to 14.0	590 (90)	66 (a: 33, b: 33, 10%)
9 g/d	440	−41.4 to 8.6	422 (96)	18 (a: 8, b: 10, 4%)
6 g/d	344	−34.4 to 15.6	318 (92)	26 (a: 17, b: 9, 8%)
All levels	1440	…	1330 (92)	110 (8%)

The prediction interval of UNaV to correctly classify recorded sodium intake is ±25 mmol of the average difference between recorded sodium intake and UNaV. UNaV samples within and outside this prediction interval are counted. Misclassified UNaV samples above the prediction interval (a), below the prediction interval (b), and the percentage of total misclassifications are given. UNaV indicates urine collection for sodium excretion.

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Table 4. Agreement Between Prescribed Salt Intake Level and UNaV

Salt Intake Level	No. of Observations, n	Prediction Interval, mmol	UNaV Within Prediction Interval, n (%)	UNaV Outside Prediction Interval, n
Single UNaV sample				
12 g/d	742	175–225	318 (43)	424 (a: 109, b: 315, 57%)
9 g/d	500	125–175	234 (47)	266 (a: 48, b: 218, 53%)
6 g/d	404	75–125	229 (57)	175 (a: 50, b: 125, 43%)
All levels	1646		781 (47)	865 (53%)
Three UNaV samples				
12 g/d	712	175–225	316 (44)	396 (a: 101, b: 295, 56%)
9 g/d	480	125–175	226 (47)	254 (a: 39, b: 215, 53%)
6 g/d	384	75–125	216 (56)	168 (a: 45, b: 123, 44%)
All levels	1576		758 (48)	818 (52%)
Seven UNaV samples				
12 g/d	656	175–225	292 (45)	364 (a: 86, b: 278, 55%)
9 g/d	440	125–175	203 (46)	237 (a: 35, b: 202, 54%)
6 g/d	344	75–125	190 (55)	154 (a: 39, b: 115, 45%)
All levels	1440		685 (48)	755 (52%)

The prediction interval of UNaV to correctly classify prescribed salt intake is ±25 mmol of prescribed intake level. UNaV samples within and outside this prediction interval are counted. Misclassified UNaV samples above the prediction interval (a), below the prediction interval (b), and the percentage of total misclassifications are given. UNaV indicates urine collection for sodium excretion.

formula (0.71).[18] The authors concluded that the Kawasaki formula is acceptable for population studies. Our data suggest that the putative gold-standard 24-hour UNaV method to assess an individual's salt intake should be applied and interpreted with caution. The Kawasaki formula would be even less precise.

In epidemiological studies, such as the international study of electrolyte excretion and blood pressure INTERSALT,[8] where >10 000 24-hour UNaV samples were collected, in population surveys[7] or in nation-wide salt-reduction programs,[19] calculating population-mean UNaV would result in reliable information on average salt intake in a given population. However, the Mars flight simulation data also imply that infradian rhythmical variability represents a systematic error when single 24-hour UNaV collection is used to representatively classify salt intake in individual persons. Tracking their fate at a later date on the basis of such a classification could lead to spurious results. The physiological disagreement between daily sodium intake and excretion represents a systematic error, which may misclassify 3-g differences in salt intake in 50% of the persons (coin flip), regardless of the study population size.

Our findings on sodium storage in the body raise questions about how existing epidemiological evidence is interpreted. The current discussions on the U-shape association between urinary sodium excretion and cardiovascular disease rely on the traditional steady-state assumption that low urinary sodium excretion invariably reflects low salt intake.[9,17,20–24] However, the agreement between salt intake and urinary sodium excretion has never been tested on the long-term in humans with known cardiovascular or renal disease. Recent evidence suggests that patients with confounding cardiovascular disease, renal disease, infectious disease, or older persons apparently store large amounts of sodium in their bodies.[25–28] Individuals with increased total body sodium storage and reduced renal sodium excretion could be easily misclassified as individuals with low salt intake. The resulting selection bias could lead to erroneous conclusions.

Well-known physiological factors that account for natural differences between sodium intake and sodium excretion are extrarenal sodium losses via skin and intestines. We could not measure extrarenal sodium losses on a day-to-day basis in our sodium balance studies. Also, differences between prescribed salt intake, recorded salt intake, and actual salt intake will alter the agreement between sodium intake and excretion. However, the mean difference between recorded sodium intake and UNaV in our study was 12±39 mmol/d. This finding quantitatively suggests that steady-state sodium balance was achieved at extrarenal sodium losses that were normal for healthy humans living under thermoconstant conditions. We conclude that biological variability of UNaV around this steady-state level characterizes long-term sodium homeostasis in humans.

Limitations

Our study necessarily has limitations. We studied only men, less than half the general population. Our data are confined to 9 whites and 1 Asian. We have no data on other ethnic groups. Conceivably, persons willing to subject themselves to a Mars simulation flight have attributes different from other humans and are not generalizable, although we do not believe that to be the case. Finally, our salt modifications were confined to altered food, as relevant for the general population and as being attempted at the population level in Great Britain with variable success.[29,30] Administration of salt in the form of slow-release capsule formulations could conceivably give a different result.

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Perspectives

Our data question the currently accepted set of tools used for clinical and epidemiological investigation of salt intake. Alternative approaches, such as direct noninvasive measurements of tissue sodium content in humans, may provide more concise information on the relationship between salt and health.[25–28,31,32] Effective and conclusive intervention studies may require particularly unique environmental situations[33] or nationwide prescribed salt reduction in processed foods.[29] Should an intervention study be entertained to test the notion that salt reduction lowers hard end points, the long-term physiological regulatory patterns of sodium balance could be carefully considered in the design of such an intervention.

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Disclosures

None.

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Novelty and Significance

What Is New?

- We recently reported findings that when humans ingest a constant salt intake (6, 9, and 12 g/d salt), the urinary sodium excretion over 24 hours (UNaV) is not constant, but instead varies around the intake in an infradian circaseptan rhythm. We reasoned that this varying daily urine collection for sodium excretion (UNaV) would confound the use of a single UNaV to estimate salt intake and to separate these levels of intake. We show that a single UNaV is no better than a coin flip or 50% in identifying these levels of salt intake, 3 UNaV collections increases precision to about 75%. A full 7 samples are necessary to achieve 90% correct estimates of salt intake.

What Is Relevant?

- The 24-hour UNaV is the gold standard for establishing salt intake. Currently, the Kawasaki formula (correlation about 0.7) that relies on a spot urine is used. Such methodology may suffice for large population studies, where population means are compared. However, for individual determinations as used clinically, or in intervention trials where the fate of individuals is the end point, single UNaV lead to spurious conclusions.

Summary

We observed that at fixed salt intake, 24-hour UNaV varies in a circaseptan pattern. This endogenous rhythm makes a single UNaV worthless for separating 3-g differences at salt intakes of 6, 9, or 12 g/d. Instead, 3 to 7 collections would be necessary for better agreement between salt intake and salt excretion. This information is important when using 24-hour UNaV clinically or in designing intervention trials.

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Online Supplement to

Agreement between twenty-four hour salt ingestion and sodium excretion in a controlled environment

Running head: *Urine sodium to estimate salt intake*

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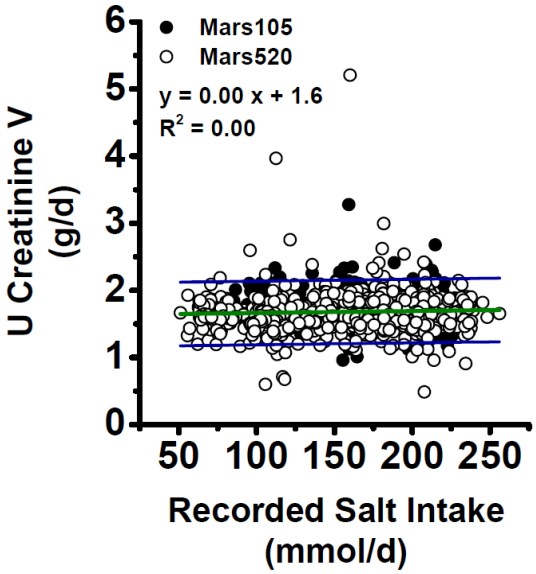
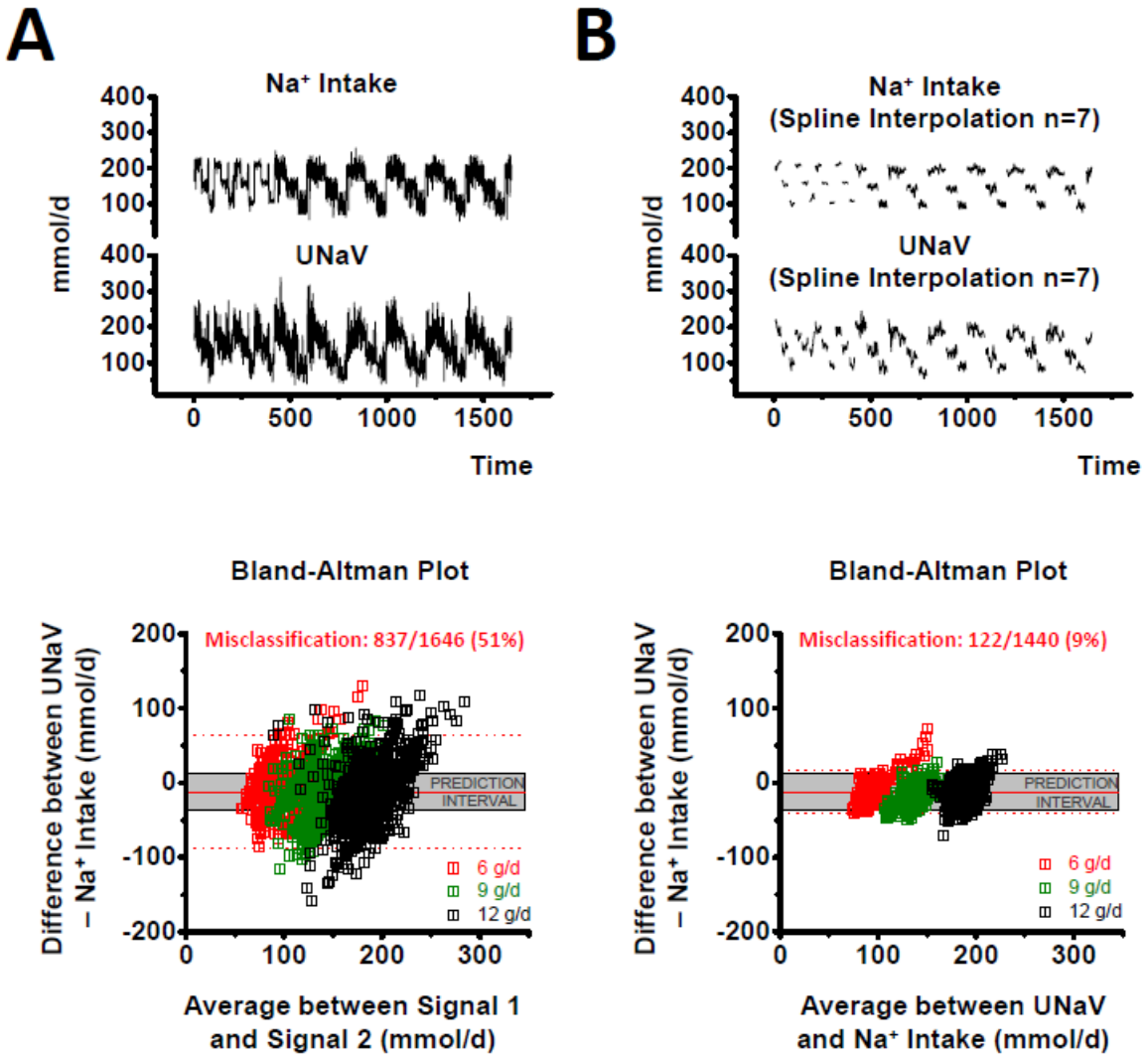
Key words Salt, sodium, salt intake, urine sodium, hypertension, cardiovascular risk

Table S1. List of offered servings, completely consumed servings, completely rejected servings, and incompletely consumed servings during the Mars105 and the Mars520 study.

Subject	Study	Offered servings	Completely consumed servings	Completely rejected servings	Incompletely consumed servings
11	105 days	1,659	1,399 (84.3%)	258 (15.6%)	2 (0.1%)
12	105 days	1,584	1,491 (94.1%)	86 (5.4%)	7 (0.4%)
15	105 days	1,526	1,348 (88.3%)	166 (10.9%)	12 (0.8%)
16	105 days	1501	1,476 (98.3%)	18 (1.2%)	7 (0.5%)
51	205 days	3,446	2,699 (78.3%)	743 (21.6%)	4 (0.1%)
52	205 days	3,758	3,027 (80.6%)	684 (18.2%)	47 (1.3%)
53	205 days	3,313	2,740 (82.7%)	570 (17.2%)	3 (0.1%)
54	205 days	3,316	2,726 (82.2%)	567 (17.1%)	23 (0.7%)
55	205 days	3,697	2,738 (74.1%)	933 (25.2%)	26 (0.7%)
56	205 days	3,479	2,991 (86.0%)	469 (13.5%)	19 (0.5%)
Sum:		**27,279**	**22,635 (83.0%)**	**4,494 (16.5%)**	**150 (0.5%)**



Online Supplement S1. Relationship between recorded Na^+ intake and 24-hour creatinine excretion in urine (UCreatinineV). Creatinine excretion was constant over all salt intake levels.



Online Supplement S2. Analysis of agreement between two parameters by transferring time series signals into Bland-Altman-Plots. *Panel A:* Signal 1 depicts the 1646 available supporting points of recorded sodium intake, and Signal 2 depicts the 1646 available supporting points of 24-hour sodium excretion in the urine. Transfer of both signals into a Bland-Altman-Plot and linear regression of the scatter points shows that Signal 1 (Intake) is in average 12 mmol/d higher than Signal 2 (Excretion). The plot also shows the remarkable

differences between individual supporting points of Signal 1 and Signal 2. A prediction interval to accurately predict Signal 1 (Intake) by Signal 2 (Excretion) is defined as ± 25 of the mean difference between the two signals. The scatter points which lie outside this prediction interval represent the number of misclassifications. Every other UNaV fails to predict recorded sodium intake within this ± 25 mmol (± 1.5 g) range. *Panel B:* Same Signal 1 (recorded Na^+ Intake) and Signal 2 (UNaV) after moving average calculation of each signal by averaging 7 consecutive supporting points. This moving average reduces the variability of Signal 1, and technically reduces the number of supporting points to 1440. Transfer of the time series into a Bland-Altman-Plot does not change the average difference between Signal 1 and Signal 2, but reduces the variability in both signals and increases the agreement between Signal 1 and Signal 2, thereby reducing the number of misclassifications. Red solid line: regression line; red dotted line: upper and lower confidence level.